SCIENTIFIC CRITERIA DOCUMENT FOR MULTIMEDIA STANDARD DEVELOPMENT NO. 01-90

N-NITROSODIMETHYLAMINE

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ABSTRACT

N-nitrosodimethylamine (NDMA) is a stable organic compound that has the potential to leach into and persist in groundwater supplies. It is an inadvertent by-product of some industrial processes, employing nitrites or amines under acidic conditions. Long term exposure to NDMA has induced liver cancer in several animal species. NDMA has been detected in water, municipal sewage, and air at certain locations in Ontario. The Interministry NDMA Expert Committee has prepared a scientific criteria document to be used in setting environmental standards. The Committee used a risk assessment approach consisting of four phases: hazard identification, exposure assessment, dose response assessment and risk characterization. A multimedia approach was used to assess exposure. The Committee took human health to be the critical endpoint for environmental exposure to NDMA. Given that appropriate epidemiological data are not available for humans, data from animal studies, specifically those of the British Industrial Biological Research Association (BIBRA) study, were used to assess risk of exposure. The Weibull mathematical model was used to estimate cancer potency. Multimedia assessment of environmental exposure to NDMA indicated that the greatest source of human exposure is food. Health-based guidelines for ambient air and water were developed for a negligible lifetime cancer risk. Levels of 0.07 and 0.7 ng/m^3 in air are associated with risk levels of 10^{-6} and 10^{-5}, respectively. Levels of 0.9 to 9 ng/L in drinking water are associated with risk levels of 10^{-6} and 10^{-5}, respectively.

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EXECUTIVE SUMMARY

This scientific criteria document reviews the available literature on the toxicity of and environmental exposure to N-nitrosodimethylamine (NDMA). NDMA is an organic compound that belongs to a family characterized by the N-nitroso functional group (-N-N=O). It is a stable liquid at ambient temperatures. In deriving guidelines for NDMA, a four step process was used: hazard identification, exposure assessment, dose response assessment and risk characterization. A multimedia approach was used to assess exposure.

NDMA is no longer used commercially. It is an inadvertent byproduct of certain industrial processes using amines or nitrites under acidic conditions. NDMA may occur in emissions from rubber manufacturing, leather tanning, pesticide manufacturing, or food processing plants. Low levels have been found in municipal sewage treatment plants.

In Ontario, low levels of NDMA have been found in the air near industrial locations in Elmira. A limited survey of five Ontario cities found no detectable levels of NDMA. Therefore current exposure levels through ambient air are below the detection limit of 2 ng/m^3. In a limited survey of West Central Ontario, the Drinking Water Surveillance Program (DWSP) detected NDMA in drinking water at Elmira, Cayuga, and Ohsweken (detection limit 2-10 ng/L). The DWSP has not found NDMA at over 40 other locations, using a detection limit of 50-500 ng/L. Very low levels have been obtained at some municipal sewage treatment plants.

The acute and chronic effects of NDMA exposure have been studied in many species of animals. Short term studies have shown that NDMA is moderately toxic, as measured by LD_{50}, to laboratory, domestic, and wild mammals. Its acute toxicity to aquatic biota is low. Long term studies have shown that hepatotoxicity is the main systemic effect of NDMA.

Genotoxic studies have demonstrated that NDMA is strongly mutagenic in most test systems although it requires metabolic activation. NDMA's mutagenic action is believed to arise through the formation of an active methylating intermediate that reacts with DNA to form DNA adducts.

NDMA is a potent non-threshold carcinogen. It is carcinogenic in a wide variety of animal species, both mammalian and non-mammalian. Tumour formation occurs primarily in the liver, kidney, and respiratory tract. The EPA (U.S. Environmental Protection Agency) and the IARC (International Agency for Research on Cancer) have classified NDMA as a probable human carcinogen. Carcinogenicity was identified in the report as the most sensitive endpoint. Human health is taken to be the critical endpoint in evaluating the risks of NDMA.

The environmental fate of NDMA in air, soil, and water is assessed in the report. NDMA is not expected to adsorb to particulates in any of these media because of its low partition coefficient. Its high vapour pressure ensures that it will remain in the vapour phase in air where it is rapidly photolyzed in daylight. On soil surfaces, photolysis and volatilization processes would lead to its rapid degradation. However, NDMA's high water solubility and low partition coefficient would make it highly mobile in soil with the potential to migrate into groundwater supplies. Dissipation beneath the soil surface is expected to be a slow process effected by microbial degradation. NDMA therefore has the potential to persist under the soil and in groundwater

supplies. NDMA is subject to a slow photolytic degradation in surface waters, but its Henry's law constant suggests that volatilization would be relatively insignificant. Bioaccumulation is expected to be unimportant.

The multimedia approach, which considers total exposure from all environmental media, was used to evaluate human exposure to NDMA. Occupational exposure was not considered. The lack of province-wide monitoring data and of suitable algorithms for apportioning risk precluded a detailed/quantitative multimedia exposure assessment. The greatest source of human exposure is food, with the total daily intake estimated to be about 200 ng. Intake via inhalation is believed to be less than 40 ng/day. Ingestion through drinking water is estimated to range between less than 3 to less than 15 ng per day. Dermal absorption is negligible. No data are available for NDMA in Ontario soil.

Suitable human epidemiologic data with regard to chronic exposure to NDMA are not available. Animal bioassay data must be used to assess risk of exposure. The BIBRA study (1978) is believed to contain the most appropriate toxicological data for quantitative risk estimation, because it was designed to evaluate the time to tumour model of carcinogenicity. Mathematical extrapolation models were used to determine the relationship between dose and response, using total liver tumour incidence. Three models have been used by different agencies : the Weibull model, linear multistage model, and the model-free approach. The different models generate widely varying estimates of cancer potency, largely because of extrapolation to very low dose levels.

The linear multistage model is the least conservative of the three and does not consider time to tumour development. The model free approach has been proposed by Health and Welfare Canada for risk assessment of NDMA. It is the most conservative of the three models, but it ignores a large proportion of the BIBRA data set to generate the slope factor.

The Weibull model was chosen for risk assessment because it takes into account time to tumour development and makes best use of the BIBRA data at low dose levels. It has been employed for NDMA risk assessment by the EPA, California Department of Health Services, and the Municipality of Waterloo. The slope factor obtained through the Weibull model is considered to be moderately conservative. Use of the surface area/body weight conversion factor is considered to be appropriate for low dose extrapolation from rats to humans. A cancer potency or slope factor of 51/mg/kg/day was thus obtained for humans and used to derive the exposure limits for NDMA.

Total human exposure to NDMA through all media should be kept in the range of negligible lifetime cancer risk (10^{-5} to 10^{-6}). Health-based guidelines for ambient air and drinking water associated with these risk levels have been developed. Levels of 0.07 to 0.7 ng/m^3 in air are associated with negligible lifetime cancer risks of 10^{-6} to 10^{-5}, respectively. Levels of 0.9 to 9 ng/L in drinking water also are associated with risk levels of 10^{-6} to 10^{-5}, respectively. In setting a guideline, the Ministry also considers factors, such as analytical detection limits, technical feasibility of control and economic and legal considerations.

1.0 INTRODUCTION

1.1 BACKGROUND

In November, 1989, N-nitrosodimethylamine (NDMA) was discovered in the groundwater of Elmira, Ontario. An interim drinking water guideline of 14 parts per trillion (ppt) was immediately established by the Water Resources Branch of the Ministry. Further monitoring of water and air at and near Elmira, and of the run-off from the Hagersville tire fire, indicated the presence of trace amounts of NDMA. The Hazardous Contaminants Coordination Branch (HCCB) was charged with coordinating a scientific report on the potential hazards and associated health risks from exposure to NDMA in the province.

1.2 ROLE OF THE INTERMINISTRY EXPERT COMMITTEE ON NDMA

In May, 1990, a Senior Regulatory Toxicologist was appointed to develop the scientific criteria for new standards for NDMA; in particular, to provide guidelines and associated risk levels based on human health considerations. This required a review of the available scientific literature and of existing guidelines in other jurisdictions.

An Interministry Expert Committee was created to support the Senior Regulatory Toxicologist by providing technical direction and a forum for critical review. Members included scientific experts from the Ministries of Environment, Health and Labour. Representatives from the Regional Municipality of Waterloo were also invited to participate because of their considerable experience in environmental monitoring of NDMA.

The Interministry Expert Committee was asked to recommend the scientific basis for guidelines in ambient air, water and soil, based on the potential multimedia exposure to NDMA. The terms of reference of the committee are summarized as follows:

* to review toxicological data available on NDMA, to quantify risk estimates and to develop the relevant dose response relationship;
* to evaluate the multimedia exposure to NDMA;
* to identify potential guideline numbers and the associated risk levels based on health considerations; and
* to prepare a report on the findings.

Members of the Interministry Expert Committee prepared sections of the report according to their areas of expertise. The draft report was completed in July, 1990 and forms the basis of this scientific criteria document.

The membership of the committee and its detailed terms of reference are contained in Appendix A.

1.3 THE MULTIMEDIA APPROACH TO RISK ASSESSMENT

The multimedia approach, which considers total exposure from all environmental media, was chosen to develop the scientific criteria for the NDMA standards. The approach recognizes that many persistent or ubiquitous contaminants are present simultaneously in food, air, water, consumer products, soil or dust. Traditionally, assessment and control of health risks have focused on one medium, such as outdoor air, usually the medium where the initial environmental release took place. This may greatly underestimate the actual environmental exposure and the risk to health of a given population.

The multimedia approach allows the setting of single medium standards that recognize the existence of multiple sources for a given contaminant and its multiple exposure pathways. The approach incorporates the major steps of risk assessment (Section 1.4) in the context of simultaneous exposure to all environmental media and pathways of exposure. By using this combined or total exposure assessment approach, an **allocation** assigns a percentage of the total tolerable dose to each exposure pathway, such as water or soil, allowing a numerical standard for each to be derived. Allocation forms part of the policy decision-making process of standard setting, because it considers scientific, technical feasibility and socio-economic factors, in contrast to other, primarily scientific, steps of the risk assessment process.

The multimedia approach has three major advantages over the traditional single medium approach. First, it ensures that total exposure is kept below maximum allowable intake or exposure levels. Secondly, consistent risk evaluations and decisions on negligible risk can be made for each exposure pathway. Finally, it allows for a single comprehensive scientific evaluation, thus avoiding duplication of effort (Thorpe, 1989).

1.4 APPROACH AND STRUCTURE OF THE SCIENTIFIC CRITERIA DOCUMENT

The criteria document provides the scientific basis for the development of NDMA standards for Ontario. The Ministry will establish the actual standards based on legal, policy and economic considerations and on public input.

The Interministry Expert Committee adopted a risk assessment framework to evaluate the environmental and human health risks of NDMA (National Academy of Sciences, 1983; Environmental Protection Agency, 1986). The term "risk assessment" was defined as follows:

> **Risk assessment** is the scientific evaluation of the probability of adverse consequences (either direct or indirect), and the accompanying uncertainties, to the environment and to human health, caused by physical, chemical or biological disturbances to the environment.

Thus, risk assessment means scientific risk assessment and does not address the social, economic, technically feasible, regulatory or legal aspects of the environmental management of NDMA. The committee reviewed all available scientific reports and literature to obtain a balanced amount of information within the time frame specified in its terms of reference.

Risk assessment consists of four phases:

* hazard identification
* exposure assessment
* dose response assessment
* risk characterization

Phase 1 - Hazard Identification

Hazard identification is the preliminary identification of the potentially adverse environmental and health impacts of a physical, chemical or biological disturbance to the environment. It includes ascribing a level of potential concern to the impacts, such as identifying the most sensitive plant or animal species or the most sensitive toxic endpoint upon exposure.

Phase 2 - Exposure Assessment

Exposure assessment is the qualitative and quantitative determination, or estimation, of the magnitude, frequency, duration and route of exposure of a particular physical, chemical or biological disturbance to the environment. It delineates the major pathways of exposure (e.g., air, water, food); the levels of exposure from each pathway ; and the total exposure of the given population from all pathways that contribute to the health risk of concern. Data for exposure assessment may be obtained from monitoring studies of the contaminant and from dynamic modelling of its environmental fate.

Exposure assessment extends to an evaluation of the uncertainties associated with the determination or estimation. Individual exposures to single environmental media should be evaluated in the context of an integrated multimedia assessment of total exposure.

Phase 3 - Dose Response Assessment

Dose response assessment is the determination of the relationship between the magnitude of exposure from different exposure routes and the probability of the occurrence of environmental or health effects. It encompasses an assessment of the uncertainties associated with this determination.

Phase 4- Risk Characterization

Risk characterization is the integration of the exposure and dose response assessment to provide a description of the nature and magnitude of the risk and of the associated uncertainties. It includes the evaluation of the contribution from different exposure routes and media to the overall risk.

The scientific criteria document reviews and summarizes the available information under each of the four phases of risk assessment.

2.0 HAZARD IDENTIFICATION

Hazard identification is the qualitative identification of the potentially adverse impacts of a given physical, chemical or biological disturbance to the environment. Hazard identification of N-nitrosodimethylamine (NDMA) required a review of its chemical and physical properties and sources (Section 2.1); its toxicology (Section 2.2); its effects on human health (Section 2.3); and its impact on the environment and associated organisms (Section 2.4).

2.1 CHEMICAL AND PHYSICAL INFORMATION

2.1.1 Chemical and Physical Properties

N-Nitrosodimethylamine (NDMA) (CAS Registry Number 62-75-9) is an organic compound with the chemical formula $C_2H_6N_2O$. Its chemical structure is given in Fig. 2.1a.

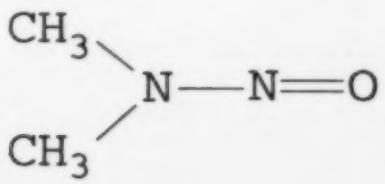

Fig. 2.1a Chemical Structure of NDMA

NDMA belongs to a class of chemicals known as N-nitroso compounds, characterized by the N-nitroso functional group (-N-N=O); and to the family of nitrosamines which, in addition, possess an amine function (-NR_2, where R is H or an alkyl group).

NDMA is the simplest dialkylnitrosamine (Molecular weight 74.08). Its chemical and physical properties are listed in Table 2.1.

NDMA is a chemically stable liquid at ambient temperatures. Two of its properties are significant in terms of its environmental impact: its high water solubility and its low octanol/water partition coefficient. This means that NDMA is not expected to bioaccumulate or to become trapped in soil, but NDMA does have the potential to leach into groundwater supplies.

Table 2.1a: Chemical Identity of NDMA*

Chemical Name	Methanamine, N-methyl-N-nitroso
Synonyms	N-nitrosodimethylamine (NDMA); dimethylnitrosamine (DMNA or DMN)
Chemical Formula	$C_2H_6N_2O$
Chemical Structure	$(CH_3)_2N\text{-}N{=}O$
CAS Registry Number	62-75-9
NIOSH RTECS Number	IQ0525000

Table 2.1b: Chemical and Physical Properties of NDMA

Property	Value
Molecular Weight	74.08
Melting Point	-50°C (estimated)
Boiling Point	154°C
Partition Coefficient log Kow (octanol/water)	-0.57
Vapour Pressure	2.7 mm Hg (20°C)
Henry's Law Constant	2.63×10^{-7} atm-m^3/mol (25°C) (estimated)
Solubility	3978 mg/L**

*Cited from ATSDR, 1989.

**Calculated from QSAR Database.

2.1.2 Mechanisms of Formation of NDMA

Nitrosamines are formed by the nitrosation of amines, usually under acidic conditions. NDMA is formed by nitrosation of dimethylamine, the simplest secondary amine.

The chemistry of nitrosation has been extensively studied and reviewed, particularly under acidic conditions (Ridd, 1961; Mirvish, 1975; Challis, 1981 ; IARC, 1982). Amines are readily nitrosated in acidic aqueous systems by various active intermediates formed from the nitrite ion, in particular, the nitrosating agent N_2O_3 (Figure 2.1b). The reaction best occurs a pH level of 3.0-3.5. Nitrosamines may thus be formed in the body by the reaction of amines with nitrite under acidic conditions in the stomach (Leaf, 1989).

Nitrosation of amines can also occur under non-acidic conditions (Challis and Kyrtopoulos, 1976; Challis and Shuker, 1979). Under neutral or alkaline conditions, the nitrosating agent, N_2O_3, cannot be formed from nitrite, but can occur as a result of the reaction of nitric oxide (NO) with oxygen (Figure 2.1c). This means that nitrosation may occur elsewhere in the body under neutral conditions through enzymatic processes involved in the production of nitric oxide (Leaf, 1989).

Other agents present in body fluids, such as the chloride ion, may promote nitrosation (Leaf, 1989). However, nitrosation can be inhibited by compounds that scavenge nitrosating agents and convert them to innocuous products. Such inhibitors include amino acids, a-tocopherol and ascorbic acid (Vitamin C).

2.2 TOXICOLOGY OF NDMA

In assessing the toxic effects of NDMA, one must consider the results of both acute, or short term, exposure and chronic, or long term, exposure. The route of exposure (inhalation, ingestion or dermal) may also be significant.

Few data on the effects of NDMA on human health are available. An examination of NDMA's mammalian and genetic toxicology allows an estimation of the potential impact on human health.

NDMA's toxicology is the subject of a number of comprehensive reviews (IARC, 1978; EPA, 1980; California Department of Health Services, 1988; ATSDR, 1989). The following sections summarize the major findings on the toxicology of NDMA. Additional details are contained in Appendices B to D.

$$NO_2^- + H^+ \rightleftharpoons HNO_2$$

$$HNO_2 + H^+ \rightleftharpoons H_2NO_2^+ \rightleftharpoons H_2O + NO^+$$

$$2HNO_2 \rightleftharpoons N_2O_3 + H_2O$$

$$HNO_2 + HX \rightleftharpoons NOX + H_2O$$

Figure 2.1b Nitrosating Species from Nitrite in Acidic Conditions.

$$2NO + O_2 \longrightarrow 2NO_2$$

$$2NO_2 \rightleftharpoons N_2O_4 \xrightarrow{H_2O} NO_2^- + NO_3^- + 2H^+$$

$$NO + NO_2 \rightleftharpoons N_2O_3 \xrightarrow{H_2O} 2NO_2^- + 2H^+$$

Figure 2.1c Nitrosating Species from Nitric Oxide in the Presence of Oxygen.

2.2.1 Mammalian Toxicology

The target organ for acute and chronic exposure to NDMA is the liver. Acute exposure to toxic levels of NDMA has resulted in a characteristic haemorrhagic necrosis in several animal species. Numerous chronic exposure studies have shown that NDMA is carcinogenic in a wide variety of species.

NDMA is readily absorbed in mammals via all three routes of exposure, inhalation, ingestion or skin contact. Little information on the extent of absorption is available, except for ingestion, which appears to be rapid in several species.

Once absorbed, unmetabolized NDMA is rapidly distributed to the main organs in mice, rats and dogs. It is extensively metabolized so that virtually no unchanged NDMA is excreted in the urine. Systematic clearance occurs with the mean elimination half life estimated to range between less than 30 to 73 minutes.

NDMA requires metabolic activation to be toxic to the body. Two metabolic mechanisms have been proposed: a denitrosation mechanism and a demethylation mechanism (Appendix B). Both pathways can occur in rat and human tissues (Yoo *et al.*, 1987; Yoo *et al.*, 1988; Yoo *et al.*, in press). The demethylation pathway results in the production of a reactive methylating intermediate that leads to methylation of various molecules in the cell, including DNA. Formation of DNA adducts is believed to be an important mechanism of genotoxic action.

Further details on the pharmacokinetics and metabolism of NDMA in mammals are contained in Appendix B.

Hepatotoxicity is the primary systemic effect of **acute exposure** to toxic levels of NDMA in all animal species tested. A characteristic haemorrhagic necrosis may occur following exposure to acutely toxic single doses or repeated doses over short durations. Humans exposed to toxic levels of NDMA by inhalation or ingestion also suffered liver damage. The mechanism of NDMA-induced liver toxicity is not well understood but may be related to the alkylation of cellular protein (Barnes and Magee, 1954; Magee *et al.*, 1976; Diaz Gomes *et al.*, 1981, 1983; Martino *et al.*, 1988).

Data on the acute toxic dosage level are available only for rats. Oral LD_{50} values of 23 and 40 mg NDMA/kg have been reported for pregnant and non-pregnant rats, respectively (Nishie 1983; Druckrey 1967). LD_{50}'s for a single dose administered by intraperitoneal injection are consistent with these findings: 26.5 mg/kg in rats (Barnes and Magee, 1954); 42.7 mg/kg in rats (Heath, 1962); and 19 mg/kg in mice (Friedman and Sanders, 1976).

Chronic exposure to NDMA in the form of repeated oral doses resulted in decreased survival in rats, mice and all other species tested. Liver tumours were the predominant effect following chronic exposures.

In general, oral doses between 0.1 to 5 mg/kg/day have produced death in animals ranging between several days to several months of exposure. Variations in lethal doses depended more on interspecies differences than on frequency or method of oral treatment.

Similarly, hepatotoxic doses occurred in the same range for all species with variations attributable more to interspecies differences. For instance, the mink species showed great sensitivity to NDMA's toxic effects. However, few studies have delineated dose-response relationships and appropriate information regarding threshold levels for hepatotoxicity is not available. The carcinogenicity of NDMA is discussed in Section 2.2.4.

Little information is available for other systemic effects in mammals, because most studies concentrated on hepatotoxicity or liver cancer. Non-hepatic systemic effects observed in rats or mink include gastrointestinal haemorrhage and congestion of several organs (kidney, lung, heart, spleen). The prevalence of the effects cannot be determined from existing data.

2.2.2 Genetic Toxicology

The literature on the genotoxicity of NDMA is extensive. Indeed, NDMA is considered to be a model mutagen and DNA-alkylating agent and is often used as a positive control in research studies. However, unlike N-nitrosoureas, which are direct-acting mutagens, NDMA is only mutagenic and reactive with biological targets after metabolic activation.

This section presents only the main aspects of NDMA's genotoxicity. A summary description of NDMA's genotoxic effects in different test systems is contained in Appendix C. It should be noted that all positive results cited required the presence of an activating system.

2.2.2.1 Detection of Lesions at the Molecular Level

Reviews on the alkylation of DNA and protein by NDMA are available (Singer and Grunberger, 1983; Pegg, 1983). NDMA was first found to be an effective methylating agent for DNA and protein through *in vivo* studies in rats (Magee and Farber, 1962). The methylation of DNA in human liver following suspected poisoning with NDMA has also been reported (Herron and Shank, 1980).

Nitrosamines, including NDMA, can react with the nitrogen and oxygen atoms present in DNA. Thirteen (13) molecular sites for NDMA methylation of DNA are possible (Singer, 1985) and twelve of these have been confirmed through animal studies. The major methylation products include: 7-methyldeoxyguanosine; phosphotriesters; O-6-methyldeoxyguanosine; 3-methyladenine; and O-4-methylthymidine.

Unscheduled DNA synthesis (UDS) as a result of NDMA treatments has been reported in a number of test systems (Appendix C). *In vitro* and *in vivo* studies in animals have demonstrated that NDMA induces UDS in various tissue and cell types.

2.2.2.2 Detection of Mutations at the Cellular Level

NDMA was the first promutagen found to have mutagenic effects in a prokaryotic organism *in vitro* (Malling, 1971). Since that time, NDMA has been reported to be mutagenic in all prokaryotic and eukaryotic test systems examined where adequate metabolic activation was provided.

Mutagenicity has been observed in direct assays involving bacteria, fungi, plants, and mammalian cells. This effect has also been found in host-mediated assays, using bacteria, fungi or mammalian cells as indicator organisms (Sankaranarayanan, 1981).

2.2.2.3 Detection of Clastogenic Effects

NDMA has been shown to induce chromosome damage in a number of test systems. Endpoints for DNA damage observed following NDMA treatment include: chromosomal aberrations in animal and plant cells; sister chromatid exchanges in animal cells; and micronuclei formation in mammalian cells.

2.2.2.4 Detection of Mutations at the Level of the Entire Organism

NDMA's genotoxicity has been examined in the progeny of treated organisms. Positive results were obtained in plants (e.g., *Arabidopsis thaliana*), insects (e.g., *Drosophila melanogaster*), and mammals (e.g., mouse spot test).

2.2.3 Reproductive and Developmental Toxicity

Transplacental transfer of NDMA has been demonstrated in pregnant rodents (Druckrey *et al.*, 1969). NDMA also has been detected in the milk of lactating rats and humans (Lakritz and Pensabene, 1984).

A number of studies have reported fetotoxicity in rats following single oral doses of NDMA. However, most of the studies do not provide adequate information on experimental design and results (ATSDR 1989). A recent study reported that a single oral dose of NDMA (20 mg/kg body weight) caused a significant decrease in fetal body weight when given on days 15 and 20 of pregnancy, but not when given on days 16 and 18. The NDMA treatments also led to maternal toxicity. The acute toxic effect of NDMA was greater in pregnant than in non-pregnant rats (Nishe, 1983). Another study treated mice with 0.02 mg NDMA/kg/day in drinking water for 75 days, covering the periods of preconception, gestation, and lactation. An increased incidence of perinatal mortality was observed, but no histological abnormalities were found nor was there any significant increase in the time to conception (Anderson *et al.*, 1978).

No NDMA-related teratogenicity has been reported, but the quality of the relevant studies has been questioned (ATSDR, 1989). Recently, the teratogenic activity of acetoxymethylmethylnitrosamine (AMMN), a derivative of NDMA, has been described. AMMN is the acetate ester of the presumed short-lived alpha-hydroxy metabolite of NDMA. AMMN has been shown to be an effective teratogen in NMRI mice following intraperitoneal administration (Platzek *et al.*, 1983; Bochert *et al.*, 1985). This finding suggests that NDMA's potential teratogenicity requires further study.

2.2.4 Carcinogenicity of NDMA

The carcinogenicity of NDMA has received extensive review (EPA, 1980; EPA, 1988; ATSDR, 1989; California Department of Health Services, 1988). NDMA was first demonstrated to be a potent carcinogen in rats in 1956 (Magee and Barnes, 1956). Several agencies have concluded that NDMA is carcinogenic in laboratory animals (IARC, 1978; EPA, 1988).

The International Agency for Research on Cancer (IARC) has noted that NDMA is carcinogenic in all animal species tested; this includes several species of mammals, birds, fish and amphibians. NDMA induces benign and malignant tumours, primarily of the liver, kidney and respiratory tract, following its adminstration by various routes, including inhalation and ingestion. NDMA was carcinogenic following its administration prenatally or in single doses. In several studies, dose response relationships were established (IARC, 1978).

To assess the lifetime cancer risk of a substance, agencies require data from well-designed studies, which follow acceptable protocols and which expose the animals to the substance for a significant portion of their lifespan. Typical criteria for the technical adequacy of animal carcinogenicity studies are summarized in Figure 2.2 (Gold *et al.*, 1984; EPA, 1986).

Four studies have been identified that meet some or all of these criteria. They have been used for the risk assessment of NDMA by various agencies. The four studies are:

* Druckrey, 1967
* Terracini, 1967
* British Industrial Biological Research Association (BIBRA), 1978
* Lijinsky and Reuber, 1984

A number of other chronic cancer bioassay studies have been reviewed by ATDSR and the California Department of Health Services (Terracini *et al.*, 1973; Griciute *et al.*, 1981; Arai *et al.*, 1979; and Tereo *et al.*, 1978, as reviewed in CDHS, 1988 and ATSDR, 1989).

Figure 2.2 Study Criteria for Assessment of Cancer Risk*

1. Test animals are mammals.
2. Exposure to the test substance begins early in life (before 100 days for rats, mice and hamsters).
3. The route of administration should expose the whole body rather than a specific site or organ, e.g., diet, water, gavage, inhalation, intravenous or intraperitoneal injection rather than subcutaneous injection or skin painting.
4. The test substance is administered alone, rather than in combination with other chemicals.
5. Exposure is chronic, with no more than seven (7) days between administrations.
6. Duration of exposure is at least one-quarter (1/4) the standard lifespan for that species.
7. Duration of the experiment is at least one-half (1/2) the standard lifespan for that species.
8. Study includes a control group.
9. Study includes at least five (5) animals per group.
10. Surgery is not performed on the animals.
11. Pathology data are reported for the number of animals with tumours rather than the total number of tumours.
12. Results reported are original data, rather than secondary analyses of data already reported.

* Taken from Gold *et al.*, 1984

The **Druckrey study** was used as the basis for the U.S. 1980 Ambient Water Quality Criterion (EPA, 1980; Anderson *et al.*, 1983). Druckrey studied the effect of a large number of nitrosamines administered in the daily drinking water of BD rats over the animals' life spans. The mean total carcinogenic dose required to produce tumours in 50% of the animals (TD_{50}) was calculated (Druckrey *et al.*, 1967).

The **Terracini study** was used to develop an interim guideline for drinking water by Health and Welfare Canada in January, 1990 (Food Safety Council, 1980; Krewski and Van Ryzin, 1981). The study summarizes a series of separate experiments where NDMA was administered in the diet of rats.

Groups of male and female MRC Porton rats ranging in numbers from 4 to 54 animals were fed diets containing 0, 2, 5, 10, 20 or 50 ppm NDMA for up to 120 weeks. Sick animals were sacrificed. Most survivors were sacrificed at 104 weeks, although a few were kept until 120 weeks. Liver tumour incidence and mortality increased significantly with dose in female rats. Tumour incidence in female rats ranged from 7% at 5 ppm to 77% at 20 ppm. All animals in the highest dose group died before the end of the experiment; tumour incidence at 60 weeks was 83% (Terracini *et al.*, 1967).

The following work is referred to collectively as the **British Industrial Biological Research Association (BIBRA) study**. A major research program on nitrosamine carcinogenesis involving 5120 animals (rats, mice or hamsters) was commissioned by the British Ministry of Agriculture, Fisheries and Food. The original data and details of the study can be found in an unpublished report (Brantom *et al.*, 1978) and in the unpublished doctoral thesis of P. G. Brantom (The University of Surrey, Guildford, U.K., 1983). A preliminary account of the study has been published (Crampton, 1980). A statistical analysis of the dose and time relationships appeared in 1984 (Peto *et al.*, 1984).

The NDMA portion of the study involved 1800 Wistar rats (Colworth strain) and a further 480 control animals (240 males and 240 females). Fifteen dose groups of 60 male and 60 female rats received NDMA concentrations of 33 to 16,900 ppb in drinking water beginning at six (6) weeks of age. These doses correspond to approximately 2 - 1,080 ug/kg/day for male animals and 3 - 1,470 ug/kg/day for females. One-tenth (10%) of the original animals were scheduled for sacrifice after 12 months and 18 months. The remaining animals (48 each of sex per treatment) were treated for the rest of their normal lifespan of about three (3) years. Animals were checked daily for signs of illness. Each rat was palpated weekly to detect onset of liver tumours. Animals found moribund, dead or with palpable lumps were taken for autopsy and all tissues examined macroscopically. Routine sampling was confined to liver, kidney, bladder, lungs, skull and esophagus or other abnormal tissue found. Records were kept on the number of animals with tumours, time of death, and tumour histology.

Liver tumours were the major cause of death. NDMA treatment resulted in all types of hepatocellular tumours (hepatic adenoma, hepatic carcinoma, haemangioma, haemangiosarcoma, biliary cystadenoma, bile duct carcinoma, Kupffer cell tumour or uncertain types). The incidence of benign and malignant liver tumours in male rats was significantly elevated over control levels at the lowest dose administered. Biliary cystadenomas were particularly common in females over the dose range 1580 to 6340 ppb.

The incidence of hyperplastic nodules was relatively constant in all treatments and did not appear to be dose-related. Other tumour sites reported with apparently positive dose response trends were the lung and seminal vesicles.

In the **Lijinsky and Reuber study** female Fisher F344 rats in groups of 20 were given 0, 5.5 or 13 mg/L NDMA in drinking water *ad libitum* five days/week for seven months. Animals were allowed to die naturally. The reported cumulative doses were 17 and 39 mg NDMA, respectively. Mortality in treated animals was higher than in untreated controls. All major organs and tissues were examined histologically. Liver tumour incidence in untreated controls was 10% compared to 70% in rats receiving 5.5 NDMA mg/L and 85% in those receiving 13 mg/L.

2.3 HUMAN HEALTH EFFECTS OF NDMA

2.3.1 Effects of Acute Exposure to NDMA

Human fatalities have been reported either due to ingestion or inhalation of toxic levels of NDMA. Haemorrhagic, necrotic and cirrhotic alterations in the liver were observed, indicating that NDMA produces similar hepatic effects in humans and animals. Therefore it is expected that NDMA may also be hepatotoxic to humans at sublethal doses.

2.3.2. Epidemiology of Nitrosamine-related Cancer

N-nitroso compounds are capable of inducing tumours in many organs of most animal species. Humans may be exposed to N-nitroso compounds present in urban air, tobacco smoke, food and drinking water. Thus, some human cancers may have the potential of being causally related to these compounds.

Many studies are available on the metabolism and interaction of N-nitroso compounds in humans and in animal models. However, no human analytic epidemiologic studies, either cohort or case control studies, are available to review the relationship of exposure to N-nitroso compounds and cancer.

Occupational studies are able to explore the effects of specific exposures on the incidence of specific cancers. The highest human exposures to nitrosamines occur in certain occupational environments, primarily in the rubber, tire and leather industries and in foundries (Preussmann and Eisenbrand, 1984; Spiegelhalder and Preussmann, 1983). No studies are available on the occurrence of cancer related to nitrosamine exposure in these occupations. However, environmental chemicals have been implicated in a reported excess of gallbladder and bile duct tumours in rubber plant workers (Mancuso and Brennan, 1970). An occupational mortality study of California workers suggested that automotive and rubber plant workers showed similar tendencies to gallbladder and bile duct cancer; textile and metal workers showed an increase in gall bladder cancers; and textile, metal and wood-finishing workers showed an increase in bile duct cancer.

Ecological studies are carried out on populations rather than occupational groups. Such studies are not analytical, but explore the general relationship between group exposure and group cancer rates by means of surrogate exposure measures assigned to the whole population group under study. Individual values of exposure are not assigned.

Ecological studies allow examination of the potential relationship between population exposure to N-nitroso compounds and the incidence of certain cancers. Thus, nitrosamines are thought to be contributing factors in the development of human bladder cancer in those infected with *Schistosoma hematobium* (Burton, 1982); esophageal cancer in beer drinkers (Armstrong *et al.*, 1982); and gastric cancer and nitrate in water (Armstrong *et al.*, 1982).

Other investigators have explored whether NDMA metabolism might be different in those with metastatic cancer compared to those with none: a high level of NDMA might indicate defective NDMA metabolism (dealkylation). NDMA levels were determined in individuals with cancer and liver metastases. No differences in the levels of NDMA were found. However, it was noted that blood NDMA levels are not a reflection of exposure or body burden (Simenhoff *et al.*, 1982).

In conclusion, evidence of NDMA 's carcinogenicity in humans derives from ecological studies and from descriptive studies in occupations with exposure to N-nitroso compounds. Allowable concentrations in environmental media need to be derived from animal data and from models which may predict human risk. In the absence of quantitative data relating human exposure to cancer outcome, the BIBRA study was selected for the dose response assessment.

2.4 ENVIRONMENTAL TOXICOLOGY

The acute and chronic effects of NDMA intake have been studied in both plants and animals. The acute toxicity of NDMA, as measured by the LD_{50}, was found to be relatively low in aquatic animal species. However, NDMA was found to cause liver or kidney damage in both wild and domestic terrestrial animals.

Chronic exposure studies have shown that NDMA causes abnormal cell growth in certain plants and is carcinogenic in several animal species. NDMA induced cancers in the liver, kidney or blood. A dose response relationship has been established for hepatocellular carcinoma formation in rainbow trout.

A summary of the studies on NDMA's environmental toxicity is contained in Appendix D.

2.4.1 Phytotoxicity

NDMA phytotoxicity studies have been conducted both in aquatic and terrestrial vegetation (Appendix D).

NDMA is not present in plants under natural conditions (EPA, 1980). However, toxicokinetic experiments have demonstrated that lettuce and spinach plants can absorb NDMA from both

sandy soil and water after two days exposure to levels ranging between 10 to 100 mg NDMA/kg. The percentage uptake was 3.25% for lettuce and 0.38% for spinach plants (Dean-Raymond and Alexander, 1976).

In static systems, the growth EC_{50}'s for green algae and blue-green algae were determined to be 4 mg/L and 51 mg/L, respectively (Draper III and Brewer, 1979).

NDMA has been shown to have tumorigenic effects in plants as well as animals. Seeds from hybrid tobacco plants, *Nicotiana sp.*, were soaked in 1 millimolar (mM) or 10 mM (74.1 or 741 mg/L) solutions of NDMA for two days. Tumours were observed in a significant percentage of germinated seedlings after 20 days (Andersen *et al.*, 1973).

2.4.2 Terrestrial Toxicology

NDMA toxicity testing has been conducted on wild animals and on domestic birds and animals (Appendix D). NDMA has been shown to cause liver damage and to be carcinogenic in a number of these species.

2.4.2.1 Toxicity to Birds

Male Peking ducks developed anaplastic hemangiosarcomas of the liver after being fed a 50 mg/kg diet of NDMA for 9 months (McCracken *et al.*, 1973).

2.4.2.2 Toxicity to Wild Animals

The LD_{50} in foxes (*Alopex lagopus*) was determined to be 10 mg/kg body weight. Dosing foxes with 0.1, 0.2, and 1.0 mg/kg body weight for an unspecified duration, resulted in changes to the hepatic veins. Foxes exposed to 0.1 mg/kg for several years developed hemangiosarcomas in the blood vessel walls. Intermediate and high doses resulted in ascites, jaundice and liver failure. Histological investigation showed centrilobular haemorrhagic liver necrosis (Koppang *et al.*, 1981).

Mink appear to be the species most sensitive to NDMA's toxic effects, although the validity of some of the studies has been questioned (ATSDR, 1989). The LD_{50} in mink was determined to be 7 mg/kg body weight via subcutaneous injection (Koppang and Rimeslatten, 1976). In an earlier study, mink fed doses of 0.32 and 0.63 mg/kg/day died after 23-34 days of exposure. There was evidence of gastrointestinal haemorrhage and liver necrosis in the animals (Carter *et al.*, 1969). However, very few mink were tested (three per dose) (ATSDR, 1989).

Other researchers observed that mink ingesting 0.1 mg NDMA/kg/day over 321-670 days developed hemangiomatous liver tumours, occlusion of the hepatic veins and death. Hepatic vein lesions were observed in mink given 0.13-0.15 mg NDMA/kg/day for 122 days (Koppang

and Rimeslatten, 1976). In a more recent study, mink were fed a contaminated diet containing approximately 0.18 mg NDMA/kg/day. Liver necrosis and moderate kidney tubule congestion were observed and approximately 55% of the animals developed hemangiomatous liver tumours (Martino *et al.*, 1988). The findings, however, have been questioned for two reasons. First, although the length of exposure was unspecified, the mink died within two months. Secondly, the dose of NDMA received in the diet is uncertain (ATSDR, 1989).

2.4.2.3 Toxicity to Domestic Animals

Domestic animals, such as sheep, pigs, and cattle, have been used to test the adverse effects of NDMA ingestion. Oral doses of 0.16-0.70 mg NDMA/kg/day to sheep over an unspecified time period resulted in liver disease and death. However, toxic effects were not found at 0.10-0.15 mg NDMA/kg/day over 200 days (Koppang, 1974).

Oral administration of 4.1, 8.2 or 20.5 mg NDMA/kg/day to pigs for 64-105 days caused vasoobstructive disease in the liver and anaplastic changes in the kidney tubules. A 15 ppm (0.62 mg/kg/day) dietary dose, however, did not produce pathological lesions within 525 days. A weakness of the study is that only a small number of animals was tested (two per dose) (Koppang, 1974).

The same researcher also conducted an NDMA toxicity study on cattle. Calves received doses of 0.4 mg/kg/day for 54 days, 0.2 mg/kg/day for 157 days, and 0.1 mg/kg/day for 480 days. All three groups experienced occlusion of the hepatic veins but the changes were minimal in the lowest dose group. Once NDMA was removed from the diet, the calves recovered and the damage to the liver tissue was repaired. Long term ingestion of NDMA did not lead to development of tumours in the cattle. Again, the problem with this study is the small number of animals treated in each group (four per dose) (Koppang, 1974).

2.4.3 Aquatic Toxicology

Studies on NDMA's toxicity to aquatic plants and animals are summarized in Appendix D.

2.4.3.1 Toxicity to Fish

Two studies exist on the acute toxic effects of NDMA on freshwater fish. In a static system, the 96 hour LC_{50} for fathead minnow (*Pimephales promelas*) was determined to be 940 mg/L (Draper III and Brewer, 1979). The LD_{50} value for rainbow trout (*Salmo gairdneri*) via intraperitoneal injection was 1770 mg/kg (Grieco *et al.*, 1978).

Several chronic studies in freshwater fish have demonstrated that NDMA is a hepatocarcinogen. Data for saltwater fish were not considered.

Guppies (*Lebistes reticulatus*) were dosed with 100 mg/L of NDMA for 56 days in a static system and developed hepatic neoplasms (Pliss and Khudoley, 1975). Another study reported that guppies receiving 4800 mg/kg of NDMA in diet over more than 13 months, developed hepatic tumours and a leiomyosarcoma in the mesentery. The significance has been questioned, however, because the guppies were maintained in distilled water (Sato *et al.*, 1973).

A dose of 50 mg NDMA/L over 22-23 weeks induced hepatomas in zebra fish (*Brachydanio rerio*) (Aydin and Bulay, 1983).

In a 20 month study of rainbow trout, doses of 75, 300, 1200, 4800, and 19,200 mg NDMA/kg in the diet induced adenomas and adenocarcinomas in the liver (Ashley and Halver, 1968). A direct dose-related response for hepatocellular carcinoma formation was demonstrated in a more recent study where rainbow trout received 3, 200, 400, and 800 mg NDMA/kg in the diet over 52 weeks. Tumours did not form in trout receiving 3 mg/kg (Grieco *et al.*, 1978).

2.4.3.2 Toxicity to Aquatic Invertebrates

Data are available on the acute toxicity of NDMA to aquatic invertebrates (Appendix D). The literature on saltwater aquatic invertebrates was not reviewed.

The acute toxicity of NDMA to aquatic organisms is relatively low. The 96 hour aqueous LC_{50} for flatworms (*Dugesia dorotocephala*) is 1365 mg/L (Draper III and Brewer, 1979) and about 280-445 mg/L for scud (*Gammarus limnaeus*) (Draper III and Fisher, 1980). The LD_{50} value for crayfish (*Austropotamobius pallipes*) is 2250 mg/kg via injection (Alibaud *et al.*, 1985).

Chronic toxicity studies have been carried out in mussels and crayfish. Mussels (*Unio pictorum*) were exposed to 200 mg/L of NDMA in tank water for 51 days and 152 days (Khudoley and Syrenko, 1978). The mussels developed tumours of the digestive gland and hemopoietic system. Eight percent (8%) of the mussels developed tumours during the 51 day exposure and 27%, during the 152 day exposure. In another study, 200 mg/L of NDMA dissolved in tank water produced digestive gland neoplasms in 35% of the mussels over a 51 day exposure (Khudoley and Syrenko, 1977).

Crayfish (*Procambarus clarkii*) were exposed for six months to water treated with NDMA and developed hyperplasia of the tubular cells in the hepatopancreas at a dose of 100 mg/L and degeneration of the antennal gland at 200 mg/L (Harshbarger *et al.*, 1971). A recent toxicokinetic study using ^{14}C-NDMA showed the highest concentration of NDMA (8.5%) to be in the hepatopancreas 30 minutes after intravenous injection (Alibaud *et al.*, 1985).

2.4.3.3 Toxicity to Amphibians

Chronic exposure studies to NDMA have been made in newts and two species of frogs, *Rana temporaria* and *Xenopus borealis* (Appendix D).

NDMA doses of 16 g/kg body weight were injected intraperitoneally into palmate newts (*Triturus helviticus*) twice a week for three to four weeks. The newts developed liver tumours such as anaplastic and hepatocellular cancers (Ingram, 1972).

Frogs (*Rana temporaria*) were exposed to 5 mg/L NDMA in water for 63 days and 203 days. At both concentrations, the frogs developed hepatocellular carcinomas as well as adenomas and tumours of the hemopoietic system. Approximately 44% of the frogs exposed for 203 days developed tumours (Khudoley, 1977). In another species of frog (*Xenopus borealis*) exposed for 52 weeks to 400 mg/L of NDMA in aquarium water, 54% of the test animals developed liver and kidney tumours (Khudoley and Picard, 1980).

3.0 EXPOSURE ASSESSMENT

Exposure assessment requires the estimation of the route and extent of exposure to NDMA from all environmental sources. The process consists of three basic steps.

The first step includes measuring or modelling the sources, fate and pathways of NDMA to determine its distribution in all environmental media. The second step involves measuring or modelling the exposure of organisms, including humans, in the affected part of the environment. The biological changes of NDMA in the target organism, the rate of excretion, and other toxicological factors must be considered in determining the delivered dose. The final step integrates the estimated exposures from single environmental media into an integrated multimedia assessment of total exposure.

3.1 NDMA INPUTS INTO THE ENVIRONMENT

The presence of nitrosamines in the environment was not confirmed until the mid-1960's after improvements in analytical detection methods. Earlier work had concentrated on food, especially nitrate-treated foods, such as cured meats.

3.1.1 Industrial Sources of NDMA

Nowadays, NDMA is only produced in very small amounts for research purposes. NDMA was used prior to 1976 as an intermediate in the production of 1,1-dimethylhydrazine, a liquid rocket fuel, but this use has been discontinued. At one time, it also was used as: an antioxidant; an additive for lubricants; a solvent in the fibre and plastics industry; a plasticizer for rubber; and a softener for copolymers (NTP, 1985) .

NDMA is generated by the nitrosation of dimethylamine under acidic conditions (Section 2.1.2). NDMA can be formed inadvertently during industrial processes involving the use of alkylamines in combination with nitrogen oxides, nitrous acids, or nitrite salts. It may therefore be present in the emissions from certain industries.

Historically, the rubber industry, especially tire manufacturing, has been linked to nitrosamines because of the amines used in rubber accelerators and stabilizers. NDMA has been detected both in the rubber products themselves and in plant emissions to air and water.

Other industries where NDMA potentially may be generated include: pesticide manufacturing, leather tanning, fish processing, foundries and dye manufacturing. The leather tanning industry is a potential source of NDMA because of the use of dimethylamine sulphate as a dehairing agent. Metal finishing operations produce nitrosamines from ethanolamines and nitrite corrosion and oxidation inhibiters, although NDMA is not believed to be produced in significant amounts.

The cosmetics industry also uses ethanolamines as emulsifiers but again NDMA is not believed to arise.

NDMA has been detected as a contaminant or by-product in the amine salt formulations of certain pesticides, such as 2,4-D, dicamba, MCPA, and 2,3,6-trichlorobenzoic acid. A study in Canada examining samples of the pesticide 2,4-D showed that more than half contained less than 0.5 ppm (parts per million) NDMA, while 10% contained an excess of 2 ppm.

In 1980, the U.S. EPA proposed a policy for reducing human exposure to pesticide-derived N-nitroso compounds (EPA, 1980). This has led to a substantial reduction in the nitrosamine contamination of pesticides (Preussmann and Eisenbrand, 1984).

3.1.2 Environmental Sources of NDMA

NDMA may be formed by environmental processes. It may be generated in night air through the atmospheric reaction of dimethylamine with NO_x. It may also be synthesized by soil bacteria from various precursor chemicals, such as nitrate, nitrite and amine compounds (ATSDR, 1989). NDMA also may be present in municipal effluent and sewage sludge, as a result of environmental processes or industrial emissions.

3.1.3 NDMA Sources in Ontario

Continuing investigations in Ontario have found very few sources of NDMA. The only significant industrial discharges, over 1 ppb (part per billion), detected to date are from: a chemical plant producing rubber chemicals; a rubber plant producing hoses and belts; and a pulp fibre de-inking and recovery operation. Municipal sewage treatment plants sampled so far have had very low but detectable levels (a fraction of a part per billion). The only confirmed effect on ambient air quality has been within the immediate vicinity of a chemical plant producing chemicals for the rubber industry.

3.2 ENVIRONMENTAL LEVELS AND TRENDS

3.2.1 Atmospheric Environment

3.2.1.1 Fate of NDMA in the Atmosphere

NDMA has a low vapour pressure (2.7 mm Hg at 20°C). Once emitted or formed, it is expected to be in the vapour phase without partitioning to particulates in the atmosphere. Organic compounds in the atmosphere, having vapour pressures greater than 10^{-4} mm Hg are expected to exist almost entirely in the vapour phase (Eisenreich *et al.*, 1981).

In daylight, NDMA vapour is expected to degrade rapidly by direct photolysis to form dimethylnitramine. The photolytic half-life of NDMA vapour exposed to sunlight has been found to range between five (5) to 30 minutes. Reaction of NDMA with photochemically-generated hydroxyl radicals or ozone molecules is expected to be too slow to be environmentally significant (ATSDR, 1989; Hazardous Substances Databank).

3.2.1.2 Levels of NDMA in the Atmosphere

Monitored levels of NDMA in air are summarized in Table 3.2a. Most of the data from the United States (Locations 1-7) were obtained during the mid-1970's. The Ontario data (Locations 9-12) were obtained in 1990. Levels found in indoor environments have also been included (Locations 14-15).

The data clearly indicate the presence of NDMA at or near some industrial sites. NDMA also has been detected at urban sites with no known sources of nitrosamines. Unfortunately, the most extensive urban data set (Location 8: the 15 cities in California) did not indicate the nature of the sites with regard to the possible presence of point sources.

More extensive data sets, with larger numbers of samples and longer sampling times, give a more balanced view of air contamination in a given area. These data are more indicative of the long term concentrations that might prevail in an area and hence are more useful for assessing health risks.

Table 3.2a: Monitored Levels of NDMA in Air

LOCATION	Type of Location	# of sites	DATES	# of samples	Sampling Duration (hours)	MEAN (ng/m^3)	RANGE (ng/m^3)	MONIT. METHOD	REF.
1.Baltimore,MD	Industrial (on-site)	na.	1977	na.	1	11600	na.	Cryotrap/ GC/HPLC/TEA	(a)
2.Baltimore,MD	Residential (next to 1)	na.	1977	na.	1	1070	na.	Same as 1	(a)
3.Baltimore,MD	Urban/ downtown	na.	1977	na.	1	na.	30-100	Same as 1	(a)
4.Belle,WV.	Industrial (Making DMA)	na.	1976	na.	2	na.	0-980	Same as 1	(b)
5.Baltimore,MD	Urban Upwind of 1	na.	1976	na.	na.	na.	20-100	na.	(c)
6.New York,NY	Urban	na.	1976	na.	na.	800	na.	na.	(c)
7.Philadelphia,PA	Urban	na.	1976	na.	na.	91	na.	na.	(c)
8.California (15 cities)	Urban	na.	1985	7-156	na.	26 (means for 15 cities)	6-94	na.	(d)

(a) *Ref: cited in ATSDR, HSDB and CHEMFATE.* Site 1 was a manufacturing location for unsymmetrical dimethylhydrazine rocket fuel; Site 2 was a residential community next to Site 1.
(b) *Ref: cited in ATSDR, HSDB and CHEMFATE.* Site 4 was near a factory manufacturing dimethylamine.
(c) *Ref: cited in ATSDR, HSDB and CHEMFATE.* Sites 5,6 and 7 were urban areas with no known point sources of nitrosamines.
(d) *Ref: cited from an EPA database.* Number of samples ranged between 7-156 for the 15 cities for a total of 322.

Table 3.2a: Monitored Levels of NDMA in Air (Continued)

LOCATION	Type of Location	# of sites	DATES	# of samples	Sampling Duration (hours)	MEAN (ng/m³)	RANGE (ng/m³)	MONIT. METHOD	REF.
9.Ontario (5 cities)	Urban	1-2	1990	7	0.5	nd.	nd.	TAGA 6000	(e)
10.Elmira, Ont.	Industrial	16	1990	26	0.5	11	nd.-91	TAGA 6000	(f)
11.Elmira, Ont.	Industrial (on-site)	4	1990	7	0.5	72	nd.-230	TAGA 6000	(f)
12.Elmira, Ont.	Industrial	4	1990	48	24	0.3	nd.-6.7	Impinger	(g)
13.Linz, Austria	Industrial	na.	1987	363	na.	na.	10-40 (in 54 of 363 samples)	na.	(h)
INDOOR AIR CONSIDERATIONS:									
14.Indoor air	Highly smoke-polluted environment		1978	na.	na.		10000-130000	na.	(i)
15.Interior air	New cars	na.	1985	na.	na.		20-830	na.	(j)

(e) *Ref: Technical Memorandum, MOE.* Sites chosen were not near any known point sources.
(f) *Ref: Technical Memorandum, MOE.* 16 sites chosen were downwind of different sources with potential emissions.
(g) *Ref: Preliminary sampling results, MOE.* Sampling time being 24 hours, the sites were not constantly downwind of potential sources.
(h) *Ref: cited in CDHS, 1988.* Site 13 is the most heavily polluted region of Austria; only 54 of the 363 samples had detectable NDMA (det. limit: 5 ng/m3)
(i) *Ref: CDHS, 1988.*
(j) *Ref: cited in ATSDR, 1989.* Age, design and decor affect these levels.

3.2.2 Terrestrial Environment

3.2.2.1 Fate of NDMA in the Terrestrial Environment

On soil surfaces, NDMA would be removed by photolysis and volatilization. The volatilization half-life from soil surfaces under field conditions is estimated to be about one to two hours (ATSDR, 1989).

NDMA is highly water soluble and has a low partition coefficient (Section 2.1). Adsorption to suspended solids and sediments in water would not be important. Hence, NDMA is expected to be highly mobile in soil with the potential to leach into groundwater supplies. Once incorporated into subsurface soil or water, beyond the penetration of sunlight, loss by volatilization and photolysis would greatly decrease. At this point, NDMA would be susceptible to slow microbial decomposition under both aerobic and anaerobic conditions. In aerobic subsurface soil, the half-life of NDMA has been found to be about 50 to 55 days. Degradation proceeds slightly faster under aerobic conditions than under anaerobic conditions (ATSDR, 1989).

3.2.2.2 Levels of NDMA in the Terrestrial Environment

NDMA may be released into soil as a result of the land application of pesticides contaminated with NDMA or sewage sludge containing NDMA. Formation of NDMA in soils is possible under conditions which favour nitrosation of nitrosamine precursors.

NDMA has been found in soil at levels of 1-8 ug/kg (dry basis) at some sites in the U.S.A.: Belle and Charleston, West Virginia; New Jersey; and New York, NY (ATSDR, 1989; and cited in the Hazardous Substances Databank).

NDMA is a common constituent of municipal sewage sludge. NDMA was detected in the dried sludge from 14 of 15 cities located throughout the U.S.A. Levels ranged between 0.6-45 ng/g (ATSDR, 1989). Occurrence of NDMA in sewage sludge appears to be the result of the biological and chemical transformation of alkylamines in the presence of nitrite (ATSDR, 1989).

3.2.3 Aquatic Environment

3.2.3.1 Fate of NDMA in the Aquatic Environment

NDMA is very water soluble in water and estimates of its octanol/water partition coefficient are low (Radding *et al.*, 1977; Mirvish *et al.*, 1976) (Section 2.1). This means that adsorption by organic particulate matter in aqueous solution would not be an important partitioning process. Bioaccumulation also would be unlikely. The U.S. Environmental Protection Agency has estimated the bioconcentration factor (BCF) for NDMA to be 0.026.

NDMA has a relatively low Henry's law constant (2.63×10^{-7} atm-m^3/mol at 20°C). This suggests that volatilization is a relatively insignificant fate process in water (Thomas, 1982).

One study examined the fate of NDMA dissolved in Cayuga Lake water that was left open to the atmosphere and stirred constantly. NDMA persisted without change over a period of 3.5 months in the absence of light. Thus, it appears that oxidation, hydrolysis, biotransformation or biodegradation are not significant factors in affecting the fate of NDMA in water (Tate and Alexander, 1975).

Available data suggest that NDMA is subject to slow photolytic degradation in surface waters. NDMA absorbs strongly in the near ultraviolet spectral region between 330 and 400 nm (Polo and Chow, 1976). However, the data are not sufficient to calculate the half-life of NDMA in surface waters. In unlit waters, such as groundwater, NDMA would probably persist. Degradation eventually might occur through microbial action but would be a slow process.

3.2.3.2 Levels of NDMA in the Aquatic Environment

Although NDMA is no longer used industrially or commercially, it may be formed inadvertently in a number of industrial processes. It may be present in the liquid effluent from industrial sources such as rubber and tire manufacturing; pesticide manufacturing; tanneries; fish processing; foundries; and dye manufacturing (Section 3.1).

Limited data are available on the levels of NDMA detected in or near industrial discharges. Concentrations up to 940 ng/L were found in surface waters adjacent to a rocket fuel manufacturing plant in Baltimore, Maryland at a time when NDMA was being used as an intermediate (ATSDR, 1989).

In the U.S.A., NDMA has been detected infrequently in the effluent from sewage treatment plants, at concentrations up to 3 - 4 ug/L (Fine *et al.*, 1977; EPA, 1980). In Ontario, NDMA concentrations of up to 2000 ug/L have been detected in municipal sewage treatment plant effluent (unpublished MOE data). The elevated concentrations were detected at a time when the treatment plant was impacted by an industrial discharge containing NDMA. In a separate survey of sewage treatment plant (STP) effluent in Ontario, 27 of 39 samples were positive for NDMA with the maximum concentration being 0.22 ug/L (Unpublished data, 1990).

Although NDMA may form during sewage treatment, the usual background levels associated with sewage treatment plant operations in Ontario are unknown.

Few data are available on the levels of NDMA in sewage sludge. In the U.S.A., NDMA has been reported to be a common constituent of sewage sludge. Levels ranging between 0.6 - 45 ppb were found in the dried sludge from 14 of 15 cities (Mumma *et al.*, 1984).

Elevated levels of NDMA may be found in agricultural soils as a result of the application of pesticides or sewage sludge containing NDMA (Section 3.2.2). Since NDMA is water soluble, NDMA from agricultural soil could contaminate surface waters or groundwater under appropriate conditions.

GC/MS scans of 24 groundwater locations in Ontario failed to detect the presence of NDMA at a detection limit of approximately 50-500 ng/L. These results indicate that, at a detection level of 50-500 ng/L, NDMA is not detectable in a wide range of surface and groundwater in Ontario.

Data from the EPA STORET water quality data base, indicate that NDMA has rarely been detected in treated drinking water in the U.S.A. Prior to 1989, NDMA had not been detected in treated drinking water in Ontario.

As part of the Drinking Water Surveillance Program in Ontario, GC/MS scans have been conducted at over 40 locations. The detection limit for NDMA in the general screen is approximately 50-500 ng/L. All locations have been negative for NDMA with the exception of Elmira. At this site, NDMA was initially detected in two wells at a concentration of about 4.0 ug/L. Private wells outside the plume did not have detectable levels of NDMA. The municipal aquifer within the centre of the plume had levels ranging between 1300 to 2900 ppb, but near the edge of the plume the values were much lower, ranging between 0.072 to 0.087 ppb. The municipal water supply had very low levels with a maximum concentration of 0.014 ppb (unpublished data, 1990).

Following the detection of NDMA in drinking water at Elmira, more specific NDMA analyses (detection limit of 10 ng/L) were conducted at a number of water treatment plants in West Central Ontario. In Cayuga, 8 of 33 samples of treated drinking water contained NDMA, with the maximum concentration detected being 44 ng/L (unpublished data, 1990).

During the follow-up investigation at Elmira, there was a report regarding the apparent formation of NDMA at a water treatment plant on the Six Nations Indian Reserve in Ohsweken. At a time when NDMA could not be detected in the raw water entering the plant, detectable levels of NDMA up to 320 ng/L were found in the finished drinking water.

Initial investigations implicated the use of a quaternary ammonium polyelectrolyte, Q-FLOC, a coagulant aid in the drinking water treatment process. NDMA appears to have been associated with the simultaneous mixing of chlorine and the polyelectrolyte flocculant in the mixing chamber. Use of Q-FLOC at Ohsweken has been discontinued and NDMA can no longer be detected in the finished drinking water at a detection limit of 10 ng/L.

3.3 HUMAN EXPOSURE PATHWAYS

Data on the extent of human exposure to NDMA in Ontario are limited. Because many of the available sampling results were reported as non-detectable, the estimates of human exposure have been based on the analytical detection limits for air and water. The exposure estimates should be considered as an upper limit, except for food. Estimates for food are based on average levels found in Canadian food.

3.3.1 Air

Humans may be exposed by inhaling air containing NDMA. Levels in Ontario are generally non-detectable, the current detection limit being 2 ng/m^3. However, detectable amounts at the ng/m^3 level, have been found only at the Uniroyal site in Elmira.

Assuming ambient air contains less than 2 ng/m^3 NDMA and that the average inhalation rate is 20 m^3/day, the daily intake is estimated to be less than 40 ng.

3.3.2 Water

Studies indicate that NDMA may be absorbed by ingestion or through the skin in mammals (Appendix B). Humans can be exposed by ingesting drinking water containing NDMA or by bathing in such water. NDMA has been detected in Ontario drinking and surface water in certain locations near sources of industrial emissions. Generally, however, the levels are below the detection limit of 2 -10 ng/L.

Assuming tap water concentrations range from less than 2 to 10 ng/L and that the daily consumption is 1.5 L per day, the daily intake by ingestion is estimated to range from less than 3 to 15 ng.

Although no data are available to determine the dermal absorption kinetics of NDMA through human skin, the study of skin absorption in rats provides the best estimate at this time. The findings were that 0.004% of a dilute aqueous solution (6 mg/L) was absorbed over 30 minutes (Wishnok *et al.*, 1982). Since NDMA in Ontario water would be present at much lower concentrations, a 0.004% absorption rate is an appropriate conservative estimate of the dermal absorption rate in humans. This suggests that exposure through skin contact with water is insignificant.

3.3.3 Soil

No data are available to allow an estimation of exposure through contact with soil.

3.3.4 Food

Diet is an important pathway for NDMA exposure. It can contribute to intake in three ways: first, as a source of direct exposure to NDMA; second, as a source of amines and nitrite which can react together in the mouth, stomach, or intestines to form NDMA; and finally, as a source of other substances which can modulate the action and formation of NDMA in the body.

The presence of NDMA was first reported in batches of nitrite-preserved herring meal used in animal feed. The feed was associated with an outbreak of liver disease and deaths in Norwegian livestock. The toxic agent was identified as NDMA which had been formed by the interaction of the nitrite preservative with naturally occurring amines in the fish meal (Ender *et al.*,1964). The discovery led to considerable analysis of human food for NDMA and other nitrosamines. The findings have been well reviewed (Sen, 1986; Sen, 1990; Havery and Fazio, 1985; Spiegelhalder *et al.*, 1980).

NDMA has been measured in the following major food categories:

* nitrate/nitrite-cured meat products (both smoked and unsmoked);
* malt and malt-based beverages and other foods dried directly by hot flue gases;
* fish and seafood (including salted, smoked and nitrite preserved);
* other foods, mainly cheese, spices, fermented foods and some plant products.

NDMA has also been detected in some food packaging and food contact materials (Sen, 1988).

In estimating the current exposure of people in Ontario by this route, only the information on recent analyses of NDMA in Canadian or U.S. food was considered.

3.3.4.1 Levels of NDMA in Meat Products

A survey of Canadian food samples was carried out by the Health Protection Branch, Health and Welfare Canada in 1979 (Sen *et al.*,1980). A more restricted study of cured pork products followed (Sen *et al.*, 1988). Mean levels were below 1 ug/kg. NDMA was not detected (<0.1 to 0.2 ug/kg) in other related products, such as baby foods containing meats, lard, butter, margarine and cooking oils (Sen *et al.*, 1980; Ibid., 1982).

Findings are summarized in Table 3.3a. The levels found in nitrate or nitrite cured meat products (both smoked and unsmoked) ranged from less than 2 to 17 ug/kg.

Table 3.3a Levels of NDMA in Meat Products

Food Sample	Number Positive/Total	NDMA Mean	Range (ug/kg)
Bologna	3/6	0.15	<0.2 - 0.8
Ham/Pork	2/9	0.1	<0.1 - 0.4
Salami	3/9	0.5	<0.2 - 4.7
Sausages	6/20	<0.2	<0.2 - 1.2
Pepperoni	2/2	0.4	<0.2 - 0.8
Smoked Meats (spiced)	6/8	0.3	<0.2 - 2
Wieners	2/9	<0.2	<0.2 - 1.1
Corned Beef (spiced)	2/2	<0.2	<0.2
Miscellaneous Meat Products	1/7	<0.2	<0.2
Fried Bacon (lean)	42/42	1.8	<0.2 -17.2

3.3.4.2 Levels of NDMA in Malt-based Beverages and Other Hot Flue Gas Dried Foods

NDMA was first reported in beer in 1977. Subsequent work showed that NDMA was formed in malt during the drying process due to the interaction of naturally occurring amines in malt with nitrogen oxides in the hot flue gas commonly used to dry this product. Improved malt drying techniques have reduced the levels of NDMA in malt and beer significantly. In 1982, average levels of NDMA in various ales and beers in Canada and the U.S.A. were 0.4 ug/L and 0.5 - 1 ug/L, respectively (Sen *et al.*, 1982). The results of a recent survey of 194 beers (148 U.S. and 46 Canadian) available in 1988 are summarized in Table 3.3b (Scanlan and Barbour, 1990, in press).

Table 3.3b Levels of NDMA in Malt-based Beverages

Type of Beer	Number Positive /Total	NDMA Mean	Range (ug/L)
Lager	57/98	0.07	<0.05 - 0.59
Light	26/62	0.06	<0.05 - 0.59
Malt	14/17	0.12	<0.05 - 0.39
Ale	11/17	0.11	<0.05 - 0.59
TOTAL	108/194	0.074	<0.05 - 0.59

Other foods subject to direct drying methods are skim milk powder, dried soup bases, instant coffee and some cereal products (Sen and Seaman, 1981; Sen *et al.*, 1982). Levels in Canadian foods as of 1981 are summarized in Table 3.3c. NDMA was not detected (<0.1 - 0.2 ug/kg) in baby cereals, baby formula, condensed milk, homogenized milk, table cream, instant coffee or Ovaltine.

Table 3.3c Levels of NDMA in Hot Flue Gas Dried Foods

Food Sample	Number Positive/Total	NDMA Mean	Range (ug/kg)
Skim Milk Powder	11/11	0.45	0.3 - 0.7
Dried Soup Bases	2/20	0.2	<0.2 - 0.25

The levels found in malt and malt-based beverages or other foods dried directly by hot flue gases range from less than 0.05 to 0.7 ug/kg.

3.3.4.3 Levels of NDMA in Fish and Seafood

Fish and seafood products (including salted, dried, smoked and nitrite preserved) contain trimethylamine oxide, trimethylamine and dimethylamine. These amines can be nitrosated during food processing to form NDMA. Seafood products from Canada and the U.S.A. have been reported to contain up to 26 ug NDMA/kg (Sen, 1990, in press). However, most of the canned and all the fresh fish analyzed were negative for NDMA (Sen *et al.*, 1985). Results of the 1985 survey of seafood available in Canada are shown in Table 3.3d. The levels of fish and seafood, including salted, smoked and nitrite preserved, ranged from less than 0.1 to 4.2 ug/kg.

Table 3.3d Levels of NDMA in Fish and Seafood

Food Sample	Number Positive/Total	NDMA Mean	Range (ug/kg)
Canned Products (shrimp, tuna, salmon, mackerel, oysters, sardines, etc)	4/16	<0.1	<0.1
Fresh Fish (salmon, sole, perch, haddock, cod, etc)	0/10		
Frozen cod and sole fillet	1/3	0.2	0.7
Smoked Fish (mackerel, herring, cod, salmon, haddock, etc)	11/16	0.6	0.3 - 3.3
Salted/Dried Fish (herring, cod, hake, caplin, turbot, mackerel, etc)	16/18	0.6	0.2 - 4.2

3.3.4.4 Levels of NDMA in Cheese

Cheese contains secondary amines and some cheeses are processed with the addition of nitrate. Thirty-one (31) samples of different cheeses imported into Canada and the same number of Canadian cheeses were analyzed (Sen *et al.*, 1978). Levels of NDMA found are summarized in Table 3.3e. The levels in cheese ranged from less than 1 to 68 ug/kg.

Table 3.3e Levels of NDMA in Cheese

Food Sample	Number Positive/Total	NDMA Mean	Range (ug/kg)
Canadian Cheeses			
Cheddar (processed)	0/2		<1
Cheddar (mild)	1/4	5	<1 - 20
Cheddar (medium)	1/2	4.5	<1 - 9
Cheddar (old)	0/2		<1
Cheese Whiz	0/1		<1
Colby	0/2		<1
Cottage	0/2		<1
Cream cheese	1/2	8.5	<1 - 17
Gouda	0/2		<1
Misc.	0/6		<1
Mozzarella	0/1		<1
Parmesan	0/1		<1
Wine cheese	1/4	17	<1 - 68
Imported			
Blue	1/1	trace	
Brie	0/1		<1
Camembert	1/3	0.6	<1 - 2
Edam	0/3		<1
Feta	1/3	2.3	<1 - 7
Gouda	5/8	6.3	<1 - 19
Havarti	2/3	4.7	<1 - 11
Processed cream cheese	3/4	2.8	<1 - 6
Provolone	1/1	9	9
Soft ripened cheese	2/3	1.3	<1 - 2
Tilsit	1/1	6	6

3.3.4.5 Levels in Other Foods

Data are limited on the levels of NDMA in other foods available in Canada. NDMA was non-detectable in tomato ketchup, canned or fried mushrooms, apple juice and a variety of vegetable oils (Sen *et al.*, 1980). However, up to 6.7 ug NDMA/kg has been reported for soy sauce (Sen, 1990, in press).

3.3.4.6 Estimation of the Daily Intake of NDMA from Food

Estimation of the intake of NDMA in food is complicated by the fact that humans also consume NDMA precursors, such as dimethylamine, in fish and leafy vegetables, or nitrite, mainly from fresh vegetables. Nitrates from fresh vegetables also can act as precursors for nitrite. All these substances may form additional quantities of NDMA in the gastro-intestinal tract.

Dietary inputs may contain naturally occurring substances or food preservatives and additives that may affect the levels of NDMA. Known inhibitors of *in vitro* and *in vivo* nitrosation in the diet include: ascorbic acid (vitamin C); alpha-tocopherol (vitamin E); thiols; phenolic compounds such as indole-3-carbinol; caffeic acid; ferulic acid; chlorogenic acid; phloridzin and 1,2- and 1,4-dihydroxyphenols. Other inhibitors are additives and preservatives such as sulphur dioxide, bisulphite, ascorbyl palmitate, propyl gallate and butylated hydroxyanisole (Sen, 1986; Archer, 1984).

Research on the interaction of NDMA precursors and NDMA inhibitors is limited. Evidence for the formation of significant amounts of NDMA following ingestion of certain foods is inconclusive. It may be that fresh fruit and vegetables, tea, coffee or milk contain enough naturally occurring NDMA inhibitors to counterbalance the daily intake of NDMA precursors. Until further evidence is available, the daily intake of NDMA will be estimated using the mean levels of NDMA in Canadian food and data on the average food intake in Canada.

The food intake estimate presented in Table 3.3f was calculated by assuming that all food samples contain NDMA at half the stated detection limit. The available food data (Sections 3.3.4.1 to 3.3.4.5) were recalculated and are shown as the "mean NDMA level" in the table.

To account for levels of NDMA in the rest of the diet, it was assumed that NDMA was present in all foodstuffs at some level below the detection limit. Half the minimum detection limit or 0.05 ug/kg has been used to estimate this assumed quantity. The correction is shown in the "all other foods" category of the table.

The total daily NDMA intake value is estimated to be about 0.2 ug or about 200 ng/day for an average Canadian exposure. This is a minimum estimate of the average daily intake. Above average consumption of certain cheeses, cured meat products, smoked or salted fish, or malt liquor would increase NDMA intake.

The estimate of average Canadian exposure is comparable to estimates made elsewhere: U.K. - 1 ug NDMA/week (Gough *et al.*, 1978); West Germany - 1.1 ug NDMA/day (Spiegelhalder *et al.*, 1980); Japan - 0.5 ug total NOC/day (Yamamoto *et al.*, 1984); U.S.A. - less than 1 ug/day (ATSDR, 1989).

Table 3.3f Estimates of NDMA Intake in Food

Food Item	Mean NDMA Level (ug/kg)	Daily Consumption (g/day)	Daily Intake (ug/day)
Bologna	0.2	3.0[1]	0.0006
Ham/Pork	0.14	11.4[2]	0.0016
Salami			
Pepperoni	0.45	10.0[1]	0.0045
Smoked Meat			
Fried Bacon	1.8	6.6[1]	0.0119
Skim Milk Powder	0.45	6.9[3]	0.0031
Dried Soup Bases	0.29	7.7[2]	0.002
Frozen Fish	0.2	5.8[1]	0.0012
Smoked/salted Fish	0.64	0.5[1]	0.0003
Cheeses (all types)	3.7	17.5[2]	0.065
Beer (all types)	0.085	121[2]-297[4]	0.01-0.025
All Other Foods	0.05	1600	0.08
TOTAL			0.18-0.2

References: 1. Statistics Canada, 1982; 2. Nutrition Canada; 3. Ontario Ministry of Agriculture and Food, 1985; 4. Brewer's Retail, 1990

3.3.5 NDMA Intake through Tobacco Smoke

NDMA is one of the most abundant volatile nitrosamines in tobacco smoke (IARC,1985). A number of its precursors are also found in tobacco smoke: nitric oxide, nitrogen dioxide, nitrous oxide, dimethylamine and trimethylamine . The concentrations of NDMA and its precursors in mainstream smoke are given in Table 3.3g.

Table 3.3g Levels of NDMA and its Precursors in Tobacco Smoke

Smoke constituent	Level/cigarette
Nitrogen oxides	100-600 ug
NDMA	2-20 ng
DMA	1-1.2 ng
TMA	0.7 ng

NDMA is also present in sidestream smoke.

A relationship has been found in humans between inhaled nitrogen oxides and urinary excretion of NDMA (Garland *et al.*,1986). Elevated levels of NDMA were also found in mice simultaneously exposed to nitrogen dioxide (NO_2) by inhalation and dimethylamine by oral administration (Iqbal, 1984).

N-Nitrosoproline (NPRO) is considered to be an indicator of NDMA formation in the body. Smokers excrete significantly greater amounts of NPRO than non-smokers following nitrate ingestion. A correlation between salivary thiocyanate level and urinary NPRO excretion was also noted (Ladd *et al.*, 1984). It therefore appears that smoking may significantly increase the daily level of NDMA intake.

3.3.6 Endogenous Production

The endogenous formation of N-nitroso compounds, including NDMA, in both animals and humans has been an area of vigorous research over the last decade (Craddock, 1990).

NDMA has the potential to be formed in the mouth, stomach, large and small intestine or the bladder. NDMA precursors, such as nitrite and dimethylamine, can react together under low pH or acidic conditions. At higher pH or non-acidic conditions, nitrosation is catalyzed by bacteria (Section 2.1).

An important pathway of nitrite formation lies in the salivary glands. The main precursor of nitrite is nitrate which is present in the diet or can be formed in the body (Hartman, 1982; Miller *et al.*, 1984). Inhalation of nitrogen oxides may also contribute to the nitrite levels in the body (Garland *et al.*, 1986). Dimethylamine can be formed by the digestion of biological amines, such as trimethylamine, choline, and lecithin; it is normally present in gastric juice (Zeisel *et al.*, 1988).

Absorption of NDMA is rapid from most areas of formation in the body. Typically NDMA is not detected in blood, urine or faeces unless NDMA metabolism has been altered by ingestion of large amounts of precursors or inhibitors. In addition to NDMA precursors, humans may

ingest substances that inhibit or compete with the nitrosation process. Consequently, estimation of the extent of endogenous NDMA formation is difficult. Evidence of NDMA formation has been indirectly estimated in three ways:

* manipulation of NDMA metabolism with alcohol and nitrosating agents which accelerate NDMA formation (Spiegelhalder and Preussmann, 1985; Milligan *et al.*, 1986);

* use of the NPRO test (Ohshima and Bartsch, 1981); or

* construction of mathematical models of the nitrosation process in various organs of the body (Challis *et al.*, 1982; Licht and Deen, 1988).

The NPRO test involves administering large amounts of nitrate and the naturally occurring amino acid, proline. Proline is normally present in the diet. It is nitrosated to N-nitrosoproline (NPRO) by the same processes in the body that form NDMA. However, unlike NDMA, NPRO is metabolized and excreted in the urine in measurable quantities. This makes NPRO a "marker" for NDMA formation. It is assumed that the rate of NPRO formation is related to the rate of NDMA formation. Nitrosation of proline at low pH (e.g., in the stomach) occurs about 22 times faster than nitrosation of NDMA under the same conditions. However, the relative rates of nitrosation of the two compounds at higher pH (e.g., elsewhere in the gastrointestinal tract) are less clear.

The NPRO test has been modified so that no nitrate or proline is administered. Under these conditions, the amount of NPRO excreted in the urine is dependent on endogenous formation and dietary content. Published estimates of NPRO production in normal people consuming diets without added nitrate, proline or other agents affecting nitrosation or NDMA metabolism, range from 2 - 4 ug/24 hr (Wagner *et al.*, 1984; Knight, as cited in Craddock, 1990).

The NPRO test has been criticized. A recent review concluded that urinary NPRO levels varied more than ten-fold between individuals; that the major contribution to urinary NPRO was diet; and that NPRO levels were not correlated with nitrate levels (Tannenbaum, 1987). Other researchers also failed to find a correlation between urinary excretion of NDMA and the urinary excretion of NPRO (Garland *et al.*, 1986).

All three methods are indirect. It appears that endogenous formation of NDMA can occur. However, NDMA's presence has been demonstrated only under conditions where excessive amounts of precursors were administered or where the nitrosation or metabolism of NDMA was perturbed by specific substances or particular disease states. The rate of formation of NDMA in normal healthy people eating a balanced diet is unclear and will fluctuate depending on dietary composition.

4.0 DOSE RESPONSE ASSESSMENT

Dose response assessment defines the relationship between the magnitude of exposure and the probability of environmental or health effects.

The toxic endpoint of NDMA is taken to be its carcinogenicity (Section 2.2.4). Dose response assessment first requires the identification of suitable tumour incidence data sets. An appropriate mathematical extrapolation model must be selected to define the relationship between the dose of NDMA and the observed health effect.

The best estimate of risk associated with different doses of NDMA is made using either experimental or epidemiological data. Since suitable epidemiological information is not available for humans, the equivalent exposure units are determined from experimental animal data. An extrapolation is made from low dose data in long term animal bioassays to negligible risk levels in humans. The BIBRA study was chosen for dose response assessment in humans (Section 2.3).

4.1 SELECTION OF TUMOUR INCIDENCE DATA

Tumour incidence data sets were evaluated according to criteria used by established agencies. To evaluate carcinogenicity, tumour response in treated animals must be compared with concurrently matched control animals. Historical control data may be used together with concurrent control data (EPA, 1986). Treatment-related carcinogenic action can be deduced from statistically significant tumour responses in specific organs or tissues. Statistical analysis should include a test for trend; the p-value can evaluate goodness of fit.

The tumour incidence data should be separated according to organ site and type. All biologically and statistically acceptable data sets should be considered. Where more than one tumour site or type has a significantly increased incidence, the data should be pooled for an estimate of total carcinogenic risk. Data for benign tumours should be combined with those for malignant tumours unless there is evidence that the benign tumours will not become malignant.

Other proliferative lesions of the rat liver, such as hyperplastic or neoplastic nodules, have been the subject of recent reclassification (Maronpot *et al.*, 1986; NTP/NIEHS, 1989). The current position is that hyperplastic nodules should be classified as foci of hepatocellular alteration.

This report considered all liver tumours, both benign and malignant. Hyperplastic nodules appear to fall into a category of lesions that are not classifiable as tumours and therefore are not part of the toxic endpoint selected. Hyperplastic nodules did not contribute to animal mortality in the BIBRA study nor was there any significant dose-related trend in their incidence over the dose range tested (Brantom *et al.*, 1978). Thus, quantitative risk assessment was not conducted using hyperplastic nodules (Mahon, 1989).

4.2 SELECTION OF THE MATHEMATICAL EXTRAPOLATION MODEL

Risks at low exposure levels cannot be measured directly, either by animal studies or by epidemiological methods. A number of mathematical models have been developed to extrapolate from the higher doses that induce effects in experimental animals to low risk exposures.

The use of quantitative risk assessment (QRA) models has been well reviewed. QRA models have several advantages: they fit the observed animal dose response data well; they can be used for non-threshold substances or processes; and they allow a mathematical relationship to be established between the level of exposure and the degree of risk, or probability of a toxic response.

A number of problems, however, exist with the use of mathematical models. First, there is a multiplicity of models and different models may yield very different estimates of risk using the same biological data. Secondly, mathematical models can represent biological processes, such as cancer, only to a limited extent, although this, in part, merely reflects limitations in the current understanding of the cancer process. Finally, there are inherent uncertainties in extrapolating probabilities from the range of bioassay results, or measurements in the 10^{-1} to 10^{-2} range, down to negligible risk, a range of 10^{-5} to 10^{-7}. The latter values lie well beyond the realm of biological certainty.

A number of QRA models have been used to estimate the risks associated with NDMA exposure. These include the Weibull model (EPA, 1980, 1988; Anderson *et al.*, 1983; California Department of Health Services, 1988); the linearized multistage model (Krewski and Van Ryzin, 1981; CanTox, unpublished) and the model-free approach (Krewski *et al.*, in press). All three models are **conservative**: they incorporate worst case assumptions. The models have been applied to the BIBRA bioassay data to derive a cancer potency estimate or slope factor (Section 4.4).

4.3 DETERMINATION OF EQUIVALENT EXPOSURE

If suitable human data are not available, animal data must be used to estimate the excess cancer risk associated with the levels of human exposure. Dose-response relationships determined in experimental animals, which are dosed, fed and kept under controlled conditions all of their lives, must be extrapolated to humans. Several factors must be addressed in establishing a dose equivalency for cancer potency from such studies: body size, population variations, exposure conditions and mechanism of carcinogenic action.

4.3.1 Body Size

Body size is a basic factor to be considered when extrapolating from rats to humans. Fundamental physiological, biochemical and pharmacokinetic processes as well as lifespan are correlated with body size. Body size is expected to affect rates of intake of air, water and food

(inhalation and ingestion); maintenance of body heat (basal metabolism); and processes governing the fate of substances absorbed into the body (pharmacokinetics).

Interspecies dose scaling, based on body size, is complicated. It has been based on: dose/kg body weight/day; dose/unit body surface area/day; dose/level in diet, water or air; or dose/kg body weight/lifetime.

In selecting an appropriate dose scaling factor, the comparative physiological, biochemical and pharmacokinetic data available are evaluated. In the absence of appropriate data, several agencies, such as the U.S. Environmental Protection Agency, the U.S. Food and Drug Administration and Health and Welfare Canada, have used the dose/body surface area/day scaling factor for complete, direct-acting carcinogens metabolized to reactive intermediates. Basic physiological processes between species, such as breathing, basal metabolism and clearance, are compared as a fractional power of body weight.

The fractional power often lies between 0.6 and 0.8. The fractional power of 0.66 (or the cube root) relates the surface area of cylindrical or round objects to their volume. Therefore scaling according to the cube root of body weight is equivalent to scaling for surface area.

Data exist on dose and the associated effects of ingesting NDMA in diet or drinking water or inhaling NDMA (Section 2). Information on the effect of NDMA on basal metabolism does not exist. Data on the pharmacokinetics (absorption, distribution, metabolism and excretion) of NDMA are available (Appendix B). At high doses, NDMA clearly affects the lifespan by causing cancer mortality in rats and other animals.

Metabolism or biotransformation may either detoxify a chemical or, as in the case of NDMA, transform it into a reactive, toxic metabolite. In rats and humans, most absorbed NDMA is completely metabolized. Unchanged NDMA is not cleared to the urine or faeces in measurable amounts. Consequently, the toxic response to NDMA is correlated with the level and duration of NDMA metabolites, principally in the liver.

Quantitative information on the distribution, metabolic clearance and levels of NDMA metabolites in human tissue is limited and must be inferred from the pharmacokinetics of NDMA in animals. Comparable quantitative pharmacokinetic information on NDMA is available for several test animal species. However, until comparable quantitative information on humans is forthcoming, dose scaling on the surface area basis is recommended.

4.3.2 Population Variation

There is the question of genetic variability and population homogeneity. In other words, was the population of rats in the cancer bioassay typical of most rats or was there some bias in the selection and treatment of the rats rendering the results less than representative.

To address this difficulty, researchers may examine for concurrent morbidity and non-tumour causes of death. Generally, use of a 95% confidence interval in the mathematical extrapolation models will address population homogeneity problems.

4.3.3 Exposure Conditions

In most cancer bioassays, animals are exposed through a specific route of exposure, such as inhalation, under controlled conditions. The same dose is used on a daily or weekly basis and the living conditions are constant. In contrast, humans experience a wide daily fluctuation in the level of dose and route of exposure. The variation depends on the type of diet, use of alcohol or tobacco and exposure to other sources of the chemical or its precursors.

The route of exposure and the subsequent pharmacokinetics may impact on the target organs. Inhaled or dermally applied chemicals may enter the blood and circulate throughout the body before being metabolized in the liver. By contrast, chemicals absorbed from the gastrointestinal tract would pass through the liver first.

The liver appears to remove and metabolize most NDMA entering the body. Thus, use of the BIBRA data to estimate the carcinogenic potential of NDMA through ingestion is appropriate. Use of the same potency relationship for inhalation or dermal exposure may require further study.

4.3.4 Mechanism of Carcinogenic Action

The carcinogenic action of NDMA is believed to depend on its demethylation to a reactive metabolite capable of methylating DNA and other cellular molecules. Only a tiny fraction of the reactive metabolite reacts with DNA. The DNA adduct so formed, if not removed by DNA repair enzymes, may cause mutation and result in the formation of an altered liver cell. This cell may die or may give rise to other altered cells, eventually giving rise to a tumour. Consequently, carcinogenic potency may depend on the dose administered to the rat; the dose at the target organ or liver; the dose of the NDMA metabolite produced or the dose of the DNA adduct formed. Based on current information, use of the actual administered dose is appropriate.

Consideration of the mechanism of carcinogenic action has implications when selecting the tumour data, the mathematical extrapolation model and the cancer potency slope. There is limited information on the relationship between NDMA metabolism and DNA adduct formation and carcinogenesis in humans. Thus, the dose/surface area correction should be used in conjunction with the 95% confidence interval when extrapolating the experimental animal data. This should provide an adequate margin of safety to account for uncertainties in extrapolating from rats to man.

4.4 SELECTION OF THE CANCER POTENCY ESTIMATE

4.4.1 Estimates using the Weibull Model

The Weibull model is statistical and attempts to model the relationship between lifetime exposure to a substance and tumour incidence. An arbitrary distribution is assumed of the individual thresholds or times to tumour development within a treated population. A time-dependent analysis is performed. The absence of a population threshold can be calculated by allowing the minimum threshold to be zero.

The Weibull model can be used to fit data from experiments which were performed with constant dosing schedules and which had significant intercurrent mortality. This makes it an appropriate model for the BIBRA study where the dependence on longevity of treatment was unusually strong.

There is a difficulty in accounting for differences in group dosages and the pattern of times at which animals develop the tumours. The Weibull median may be used to describe how each treatment group relates to the dose.

Cancer potency is estimated from the slope of the dose response curve. Two outputs have been derived for NDMA, one by the Environmental Protection Agency (EPA, 1988), the other by the California Department of Health Services (CDHS, 1988). Slope factors were derived from the data of a 1984 study done on 4440 inbred rats (Peto *et al.*, 1984). The values of the human slope factor obtained by each agency can be shown to be comparable (Appendix F).

The U.S. EPA found the increased risk of 1 ug/kg/day over 3 years to be 7.8×10^{-3} or a slope factor for rats of 7.8/mg/kg/day. The slope factor for humans was calculated to be 51/mg/kg/day by applying the equivalent of a scaling factor for surface area. The cube root ratio of the assumed human body weight of 70 kg and the reported rat weight of 250 g was used or a body weight to surface area conversion factor of 6.5.

The slope factor is based on the assumption that a 70 kg individual drinks two litres of water per day and inhales 20 m^3 of air per day. All exposure to NDMA was assigned to either the water exposure or the air exposure and calculated independently. No obvious consideration was given to NDMA exposure from other routes, nor to the risk associated with the combined exposure from water, air and other routes.

The California Department of Health Services (CDHS) estimated the cancer potency of NDMA using the same data (Peto *et al.*, 1984). However, this agency obtained human potency estimates (q_{human}) of 16 and 12/mg/kg/day, based on female and male rats, respectively (Appendix F).

The CDHS and the EPA have interpreted the Weibull formula differently. The EPA took the CI value for all liver tumour sites (female rats), corrected for background and calculated a slope factor of 7.8/mg/kg/day for rats. A body weight to surface area conversion factor was applied to yield a slope factor of 51/mg/kg/day for humans.

The CDHS estimated the slope factor by taking the derivative of the whole expression then substituting in the approximate values suggested by Peto to derive a slope factor of

0.4/mg/kg/day for female rats (Peto *et al.*, 1984). A correction was made for the longer lifetime of control and low dose rats in the study; the lifespan could be up to four years compared with the usual two years in cancer bioassays. Finally, the body weight to surface area conversion was applied to get the human slope factor of 16/mg/kg/day (Appendix F).

If California had used Peto's values in the derivative expression a value of 6.8/mg/kg/day would have been obtained. Using the 6.5 scaling factor, the resulting human slope factor is 44.2/mg/kg/d. This suggests the EPA and California values are comparable (Appendix F).

4.4.2 Estimates using the Linearized Multistage Model

The "multistage" polynomial model, originally described in 1961, adequately fits a large body of human and animal bioassay data (Armitage and Doll, 1961). Subsequent modification has made it the low dose extrapolation method of choice for the U.S. EPA (Crump and Watson, 1979; Anderson *et al.*,1983).

The model employs enough arbitrary constants (q_i) to fit almost any monotonically increasing set of dose response data. The cancer potency or slope factor is defined as q_1^*, the upper 95% confidence limit on parameter q_1 (Appendix F). The parameters can be estimated using the GLOBAL series of computer programs, now called ToxRisk (Crump *et al.*, 1989).

ToxRisk was used to estimate q_1^* from the BIBRA data for total liver tumour incidence in female rats, excluding hyperplastic nodules. ToxRisk was run for all doses and with the upper doses dropped, to focus on the linear part of the dose response curve. The program was run with the surface area to body weight conversion factor incorporated to obtain human slope factors.

The q_1^* values obtained were comparable to the U.S. EPA Weibull method. The q_1^* for total liver tumours, based on female rat data, ranged up to 37.2/mg/kg/day depending on how the dose response curve was fitted.

4.4.3 Estimates using the Model-Free Approach

The model-free approach, formerly called the Robust Linear Model, does not use parameters. It represents a movement away from models which attempt to fit the dose response curve over most of the observable effect range and to extrapolate linearly to lowest experimental doses (Krewski *et al.*, 1990, in press).

The approach requires selection of the lowest significant dose by comparison with the zero-dose control. The 95% confidence interval is calculated and the secant approximation to the linear portion of the dose response curve is estimated. The method has a tendency to calculate slope estimates about 30% to 100% higher than the linear multistage model.

The model-free approach is the method of choice for Health and Welfare Canada. Health and Welfare Canada applied it to the BIBRA data for female rats, excluding hyperplastic nodules. The slope factor reported below was back-calculated from drinking water doses in ng/L for a

1/1,000,000 lifetime cancer risk (Material supplied by R. Burnett). Human body weight was assumed to be 70 kg and a body weight to surface area conversion factor of 6.5 was employed. Drinking water consumption was taken to be 1.5 L/day. The slope factor was found to be 93.33/mg/kg/day, a value higher than those obtained by either the Weibull or the linear multistage model. This higher value emphasizes the extreme sensitivity of the model-free method to minor but statistically significant variations in the low dose region of the bioassay dose response curve.

The model-free method has been criticized for ignoring the complete data set and thus, failing to incorporate dose response trends and time to tumour information. When applied to the data for NDMA, this results in the omission of an extensive data set. A further concern is the quality of the control data. Other historical control data for the Colworth strain of Wistar rats indicate that the controls used in the BIBRA study had a very low incidence of spontaneous liver tumours (Thorpe *et al.*, 1982 cited in EPA Risk Assessment Forum, 1986). Consequently, the significance of tumour incidence at low doses needs to be re-examined. Pending re-examination, the slope factor derived using the model-free approach must be considered preliminary.

4.4.4 Selection of the Slope Factor

The most sensitive toxic endpoint for chronic exposure to NDMA is cancer (Section 4.0). The selected bioassay for determination of the slope factor is the BIBRA study (Brantom *et al.*, 1978). All liver tumours, both benign and malignant were considered, although hyperplastic nodules were excluded. In determining equivalent exposure among species, use of the surface area/body weight conversion factor was considered to be the best option, since the understanding of NDMA's mechanism of carcinogenic action in animals and humans is incomplete.

The Weibull model, as developed by Peto, was chosen as the mathematical extrapolation model most appropriate for the extensive data set of the BIBRA study (Peto *et al.*, 1984) (Section 4.4.1). The steepest slope factor obtained was 51/mg/kg/day (EPA, 1988). This value will be used in deriving the exposure limits for NDMA.

5.0 RISK CHARACTERIZATION

5.1 RISK CHARACTERIZATION AND GUIDELINE DERIVATION

Risk characterization is the integration of the exposure and dose response assessment to provide a description of the nature and magnitude of the risk and of the associated uncertainties. It includes a determination of the major routes of exposure and of the specific population or part of the environment at risk. The magnitude and type of risk from each route of exposure is assessed and an evaluation is made of its contribution to the overall risk.

The output of risk characterization is usually given as a single point estimate of risk. In the case of NDMA, the estimate is of carcinogenic risk. However, it must be realized that some degree of uncertainty is contained in these apparently precise predictions of risk.

The question of uncertainty in risk assessment has been reviewed (Finkel, 1990). The sources of uncertainty include:

* lack of precision of scientific measurements;
* incomplete knowledge of underlying biological and environmental processes;
* variability in human and animal populations;
* assumptions and limitations inherent in predictive models; and
* random workings of chance.

Time limitations precluded systematic quantification of the attendant uncertainties of each stage of the risk assessment process. However, at each decision point, assumptions were conservative. This does not mean that worst case assumptions were always accepted. Rather, the decision, in the absence of absolute scientific data, was protective of human health. Risk estimates presented in Section 5.0 may therefore be regarded as protective of human health.

In summary, cancer is identified as the most sensitive toxicological endpoint for guideline development, with NDMA being a non-threshold carcinogen. Humans are the population at risk and may be exposed to NDMA through air, water, soil and food. Based on risk assessment, the EPA cancer potency slope of 51/mg/kg/day has been adopted to estimate risk (Section 4). The unit cancer risks or incremental lifetime cancer risks associated with unit doses of exposure to NDMA are presented in Table 5.1.

Table 5.1 Unit Cancer Risks Associated with Unit Doses of Exposure

Medium	Unit Dose	Incremental Lifetime Cancer Risk
Ambient air	1 ng/m^3	1.4 X 10^{-5}
Drinking water	1 ng/L	1.1 X 10^{-6}
Food	1 ng/kg	1.1 X 10^{-6}

Because limited data are available for Ontario and because many samples have had non-detectable levels of NDMA, estimates of human exposure are based on detection limits for air and water (Appendix E). These estimates should be considered as worst case except for food. Estimates for the latter are based on average levels in food.

Assuming ambient air contains less than 2 ng/m^3 NDMA and the daily inhalation rate is 20 m^3/day, the daily intake is estimated to be less than 40 ng/day. The incremental lifetime cancer risk is less than 3 X 10^{-5}.

Assuming that drinking water consumption is 1.5 L/day and that drinking water concentrations range between less than 2 ng/L to less than 10 ng/L, the daily intake is estimated to be range between less than 3 to less than 15 ng/day. The incremental lifetime cancer risk therefore ranges from less than 3 X 10^{-6} to less than 1 x 10^{-5}.

Due to lack of data for soil, no estimates can made at this time.

The average daily intake of NDMA from food is estimated to be about 0.2 ug or 200 ng/day, yielding an incremental lifetime risk of 2 X 10^{-4}.

5.2 HEALTH-BASED GUIDELINES FOR NDMA

Total human exposure to NDMA in air, water, diet, soil and food should be kept in the range of negligible lifetime cancer risk (10^{-5} to 10^{-6}). In view of the current exposure from food, air and water guidelines should be based on a negligible lifetime cancer risk.

The following recommendations derive from the health-based risk assessment and do not incorporate any social, economic, technical feasibility, regulatory or legal considerations. Using

the results of the risk assessment (Section 5.1), the incremental lifetime cancer risks can be calculated to determine ranges of negligible risk for each medium.

Using the cancer potency slope of 51/mg/kg/day and assuming an intake of 20 m^3/day, the incremental lifetime cancer risks from inhalation have been calculated and are presented in Table 5.2a. Thus, guidelines of 0.07 to 0.7 ng/m^3 for NDMA would be associated with a lifetime cancer risks of 10^{-6} and 10^{-5}, respectively.

Table 5.2a Levels of NDMA in Ambient Air and Associated Incremental Lifetime Cancer Risks

Level of NDMA in Air	Incremental Lifetime Cancer Risk
0.7 ng/m^3	10^{-5}
0.07 ng/m^3	10^{-6}

For a 70 kg individual consuming 1.5 L/day of drinking water, using the cancer potency slope of 51/mg/kg/day, the incremental lifetime cancer risks for drinking water have been calculated and are presented in Table 5.2b. Thus, drinking water guidelines for NDMA of 0.9 and 9 ng/L (ppt) would have associated incremental lifetime cancer risks of 10^{-6} and 10^{-5}, respectively.

Table 5.2b Levels of NDMA in Drinking Water and Associated Incremental Lifetime Cancer Risks

Level of NDMA in Water	Incremental Lifetime Cancer Risk
9 ng/L	10^{-5}
0.9 ng/L	10^{-6}

Due to lack of data, no recommendations can be made for soil at this time.

A summary of NDMA guidelines used in other jurisdictions in the U.S.A. is presented in Table 5.2c. A considerable range in guideline values is observed for both ambient air and drinking water. The lowest values are associated with an associated risk level of 10^{-6}. It is noted, in the case of drinking water, that these values lie below the current analytical detection limit of 5 ppt (Appendix E).

Table 5.2c Summary of NDMA Guidelines in Air and Water in Other Jurisdictions

Jurisdiction	Air (ng/m³)	Water (ng/L)	Associated Risk Level	Comments	Reference
Minnesota	-	14	-	-	ATSDR, 1989
US-EPA	-	0.68	10^{-6}	water and fish consumption	EPA, 1988
US-EPA	-	8000	10^{-6}	fish consumption only	EPA, 1988
US-EPA	-	1.4	10^{-6}	water and fish consumption	EPA, 1980
US-EPA	-	16000	10^{-6}	fish consumption	EPA, 1980
Kansas	0.07	1.4	10^{-6}	annual average	ATSDR, 1989
US-EPA	0.07	-	10^{-6}	-	EPA, 1988
Philadelphia	1.2	-	TLV-based	annual average	ATSDR, 1989
North Carolina	50	-	10^{-5}	annual average	ATSDR, 1989
Virginia	3000	-	TLV-based	24 hour average	ATSDR, 1989

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APPENDIX A

TERMS OF REFERENCE

INTERMINISTRY NDMA EXPERT COMMITTEE

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INTERMINISTRY NDMA EXPERT COMMITTEE

1.0 PURPOSE

N-nitrosodimethylamine (NDMA) has been detected in drinking water, ground water and surface water, and in some air samples in the Elmira area. NDMA has also been found in water and soils at the site of the Hagersville fire. In the absence of Canadian guidelines, Ontario has adopted an interim drinking water guideline to evaluate the potential health risks from these exposures and as the basis for implementing regulatory controls of the industrial source of the NDMA. Based on literature review, NDMA is also known to be present in the air, food and various consumer products. The Interministry Committee is to recommend the scientific basis for guidelines for NDMA in ambient air, water and soil, considering the multimedia exposure potential of this compound. Other nitrosamine compounds may be considered if relevant.

2.0 TASKS

1. Identify and review the most appropriate toxicological data for quantitative risk estimation and assess and evaluate the dose-response relationship for the most appropriate endpoint to protect the health of the general population.

2. Evaluate the multimedia exposure (total exposure to air, water, soil, diet and consumer products) to NDMA, and recommend how to consider these multiple exposure pathways in deriving single environmental medium guidelines for NDMA.

3. Identify potential guideline numbers and associated risk levels based on health considerations (lifetime cancer risk).

4. Prepare a short report summarizing the above and any other pertinent information which the committee considers appropriate.

3.0 TIMING

Given the urgency of need for the above information to choose regulatory control options for NDMA, the report should be completed by July 31, 1990.

MEMBERSHIP

Dr. B. Birmingham (Chairman)
Senior Regulatory Toxicologist
Hazardous Contaminants Coordination Branch
Ministry of the Environment

Ms. S. Venkataramaiah (Secretary)
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APPENDIX B

MAMMALIAN TOXICOLOGY OF N-NITROSODIMETHYLAMINE

PHARMACOKINETICS AND BIOCHEMISTRY

APPENDIX B

MAMMALIAN TOXICOLOGY OF N-NITROSODIMETHYLAMINE

PHARMACOKINETICS AND BIOCHEMISTRY

1.0 PHARMACOKINETICS OF NDMA

Toxic response to a chemical is influenced by the level and duration of its presence in the body. The pharmacokinetics of a substance are an important measure of its fate in the body.

1.1 Absorption

1.1.1 Inhalation exposure

Studies on the inhalation uptake of NDMA are limited. However, there is evidence that NDMA is excreted in the urine of rats and dogs following air exposure (Klein and Schmezer, 1984; Raabe, 1986). Inhalation of several chemicals, including NDMA, resulted in 40-54% uptake in beagles (Raabe, 1986).

Human studies, related to tobacco smoking and NDMA levels in human urine during fasting, suggest some correlation between urinary levels and nitrogen dioxide (NO_2) levels in air (Garland *et al.*, 1986; Conney *et al.*, 1986).

1.1.2 Oral exposure

NDMA is rapidly absorbed from the gastrointestinal tract of animals (Diaz Gomez *et al.*, 1977). Several studies of the effect of ethanol, vitamin C, alpha-tocopherol or added nitrates or nitrites on the urinary excretion of ingested NDMA, indicate that most ingested NDMA is metabolized before excretion (Spiegelhalder *et al.*, 1982; Garland *et al.*, 1986; Milligan *et al.*, 1986; Conney *et al.*, 1986).

1.1.3 Dermal exposure

Several studies have been reviewed (Brecher and Light, 1990). NDMA may be absorbed through rat skin and the urinary excretion pattern is comparable to that following oral administration.

NDMA can be absorbed through rat skin membrane *in vitro*. Following 30 minutes exposure, 0.004 % of an NDMA solution (concentration approximately 6 mg/L) diffused through the

membrane. At a higher concentration of NDMA (about 74 mg/L), 6% of the NDMA was absorbed (Wishnok *et al.*, 1982). Following an application of 350 ug of NDMA to the skin of rats, 0.03 % of the dose was excreted in the urine over 24 hours (Spiegelhalder *et al.*, 1982).

1.2 Distribution

Distribution is related to the rate of blood flow and tissue perfusion and determines the concentration of a chemical in various parts of the body. Tissue concentrations of absorbed chemicals in rapidly perfused tissues like the liver rise quickly.

Unmetabolized NDMA is rapidly distributed to the main organs of mice, rats and dogs, following intravenous (i.v.) administration. The systemic clearance is rapid following a single bolus dose, with mean elimination half lives of less than 30 minutes up to 73 minutes. Mean systemic clearance rates ranged from 40 to 66 mL/minute/kg, rates close to or exceeding hepatic blood flow. This suggests extra-hepatic metabolism. Generally, no unchanged NDMA was excreted (Mico *et al.*, 1985; Gombar *et al.*, 1987, 1988).

Transplacental transfer has been shown in pregnant rodents (Druckrey *et al.*, 1969). NDMA also has been detected in milk of lactating rats and humans (Lakritz and Pensabene, 1984).

1.3 Excretion

Clearance relates to mechanisms of removal, by the kidney via the urine, or the liver and gut via biliary and faecal eliminations. The term "metabolic clearance" refers to the disappearance of the parent compound due to its metabolism to intermediates.

Virtually none of the NDMA entering the body or formed endogenously is excreted in the urine, because it is extensively metabolized. Agents inhibiting NDMA metabolism do lead to increased levels of NDMA in urine. Various studies have attempted to estimate levels in human saliva, gastric juices, blood and urine as well as the factors influencing them (Ellen *et al.*, 1982; Garland *et al.*, 1986; Milligan *et al.*, 1986; Conney *et al.*, 1986; Zeisel *et al.*, 1988; Cengiz *et al.*, 1989; Dunn *et al.*, 1986).

2.0 METABOLISM AND BIOCHEMISTRY OF NDMA

Metabolism or biotransformation may detoxify a chemical or, in the case of NDMA, form a reactive, toxic metabolite. NDMA requires metabolic activation in order to be toxic to the body (ATSDR,1989). Two mechanisms have been proposed:

1. demethylation - oxygenation or hydroxylation of the alpha-carbon leads to demethylation which is catalysed by a cytochrome P450-dependent enzyme system known as NDMA-demethylase; and

2. denitrosation - also cytochrome P450-dependent.

Both pathways are shown in Figure B-1.

Demethylation results in the formation of formaldehyde, molecular nitrogen and a methylating agent. Production of the reactive methylating intermediate leads to methylation of various molecules in the cell, including DNA.

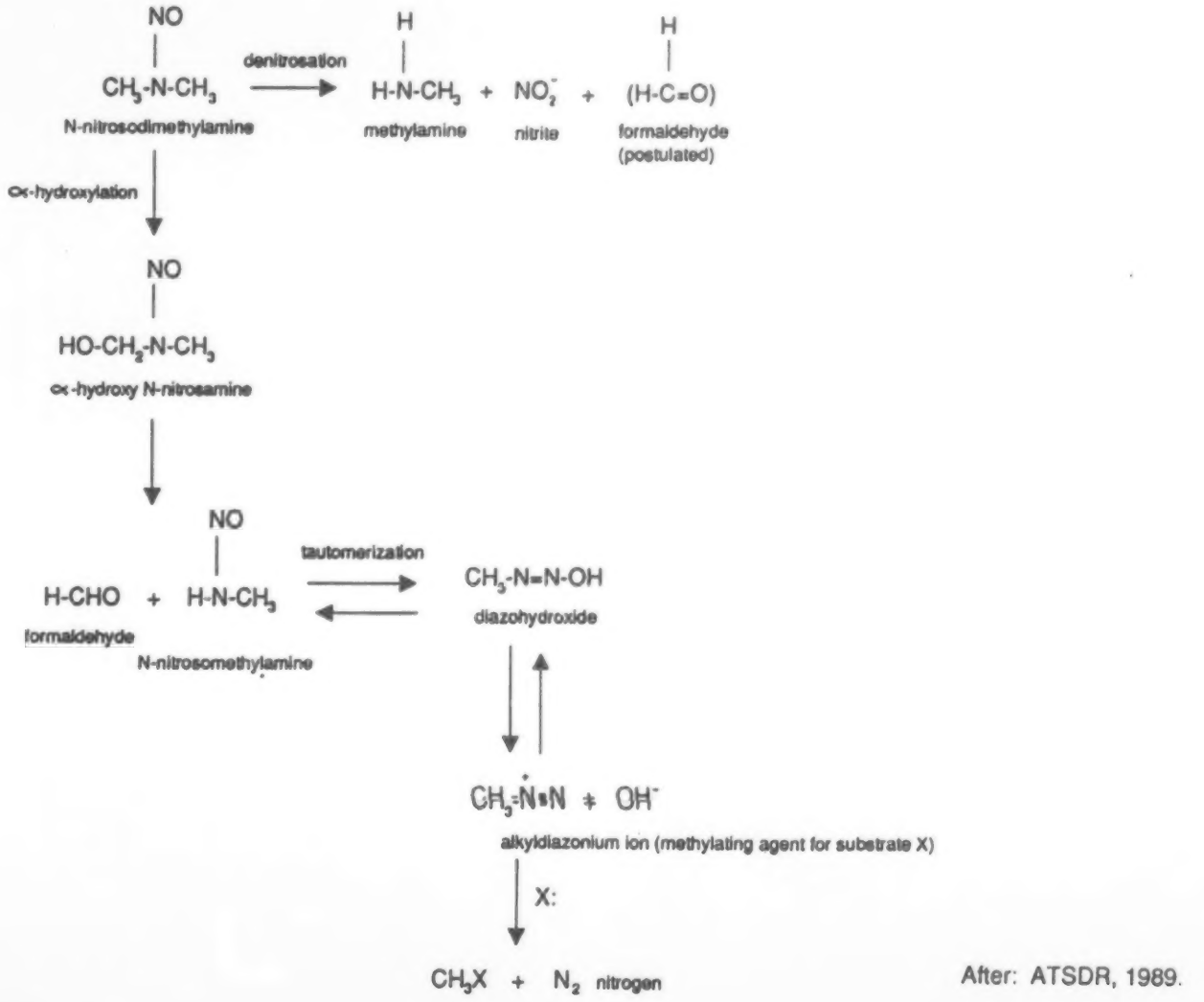
Denitrosation results in formation of nitric oxide and primary and secondary amines (mainly methylamine, dimethylamine and nitrite).

In vivo and *in vitro* studies have shown that both pathways can occur in human and rodent tissues. Human and rat liver microsomes appear to contain similar enzymes and metabolize NDMA with similar efficiency *in vitro* (Yoo *et al.*, 1987; Yoo *et al.*, 1988; Yoo *et al.*, in press).

At low concentrations cytochrome P-450$_{ac}$ may catalyse both demethylation and denitrosation. At low concentrations denitrosation accounts for less than 10% of the metabolism of NDMA. At higher concentrations, this ratio changes and a third pathway of NDMA denitrosation may exist (Keefer *et al.*, 1987).

Hepatic metabolism of NDMA is enhanced by iminopyrine, thiocyanate as well as ethanol consumption and lowered carbohydrate intake (Archer, 1984; Sato *et al.*, 1986). However, certain drugs and chemicals, such as 4-methylpyrazole, dithiocarbamates and ascorbic acid, inhibit metabolism of NDMA (Perchiballi and Hotchiss, 1989; Yoo *et al.*, 1987).

Figure B-1: Cytochrome P-450 catalysed metabolism of NDMA



After: ATSDR, 1989.

APPENDIX C

GENETIC TOXICOLOGY OF N-NITROSODIMETHYLAMINE

Table C-1. **Summary of Genotoxic Effects Observed with NDMA in Different Test Systems**

Test system	Assay	Results	Remarks
Bacteria			
Direct Assays			
E. coli	Forward and reverse mutations	+	Mutagenic only in the presence of mammalian microsomes
	Differential cell-killing in DNA-repair-deficient strains	+	Same as above.
	Preferential growth inhibition in DNA-repair-deficient strains	+	Same as above
B. subtilis	Forward mutations	+	Same as above
	Preferential growth inhibition in DNA-repair-deficient strains	+	Same as above
S.typhimurium	Reverse mutations	+	Same as above
Host-mediated assays			
E. coli	In mouse, forward and reverse mutations; in baboon, reverse mutations	+	
S.typhimurium	In mouse or rats, reverse mutations	+	
S. marcescens	In mouse, reverse mutations	+	

Test system	Assay	Results	Remarks
Fungi			
Direct assays			
S. cerevisiae	Forward mutations and cytoplasmic petites	+	Mutagenic in presence of Udenfriend's hydroxylation mixture
	Mitotic crossing-over	+	Mutagenic in presence of Udenfriend's hydroxylation mixture or mammalian microsomes
	Mitotic gene conversion	+	Mutagenic in presence of mouse liver microsomes
N. crassa	Forward mutations	+	Same as above
	Reverse mutations	+	Mutagenic in presence of Udenfriend's hydroxylation mixture
Host-mediated assays			
S. cerevisiae	In mouse or rat, mitotic crossing-over and/or gene conversion	+	
S. pombe	In mouse and rat, forward mutations	+	
N. crassa	In mouse, forward mutations	+	

Test system	Assay	Results	Remarks
Mouse lymphoma cells	Host-mediated assay	+	
L5178Y; asn$^-$	In mouse, forward mutations	+	
D. melanogaster	Recessive lethals	+	Mutagenic in all germ cells; adult feeding more effective than injection; chromosome breakage only at very high concentrations
Mammals			
Mouse	Spot tests (somatic mutations)	+	
	Dominant lethals	(+)	
Rat lymphocytes *in vivo*	Chromosome aberrations	+	
Chinese hamster ovary cells *in vitro*	Chromosome aberrations	+	Effective in presence of rat liver microsomes
Human lymphocytes	Chromosome aberrations	+	Effective in presence of mouse liver microsomes
Rat lymphocytes *in vivo*	Sister chromatid exchanges	+	
Mouse bone marrow cells *in vivo*	Sister chromatid exchanges	+	
Chinese hamster ovary cells *in vitro*	Sister chromatid exchanges	+	Effective in presence of rat liver microsomes

Test system	Assay	Results	Remarks
Mammals (cont'd)			
L5178Y cells *in vitro*	Forward mutations to TK^-	+	Mutagenic in presence of mouse/rat liver microsomes
	Forward mutations to HG-PRT^-	+	Mutagenic in presence of rat liver microsomes
V79 Chinese hamster cells *in vitro*	Forward mutations to HG-PRT^-	+	Mutagenic only after activation
Mouse bone marrow cells	Micronucleus test	+	
Human fibroblasts	Unscheduled DNA synthesis	+	Effective only in the presence of microsomes
C3H mice	Unscheduled DNA synthesis in lung, liver and kidney cells; subcutaneous injection	+	
C3H x 101 F_1 male mice	Unscheduled DNA synthesis in testis (spermatids); IP injection	-	
Plant systems			
V. faba lateral roots	Chromosome aberrations	-	
Barley seeds	Chlorophyll deficient mutations and chromosome aberrations	-	
G. max (soybean) seeds	Somatic crossing-over	+	
A. thaliana	Forward mutations		

Symbols: + = positive; - = negative; (+) = weakly positive (Sankaranarayanan, 1981)

APPENDIX D

SUMMARY OF ENVIRONMENTAL TOXICITY STUDIES ON NDMA

Table D-1: Effects of NDMA on Plants

Species	Route	Dose	Duration	Effects/Endpoint	Reference
green algae	water	4 mg/L	96 hour	EC_{50} (growth)	Draper III and Brewer, 1979
blue-green algae	water	51 mg/L	96 hour	EC_{50} (growth)	Draper III and Brewer, 1979
tobacco plant seeds (*Nicotiana sp.*)	water	74.1, 741 mg/L	2 days	tumours on germinated seedlings	Andersen, 1973
lettuce	soil	10, 100 mg/kg soil	2 days	3.25% uptake	Dean-Raymond and Alexander, 1976
spinach	water	10, 100 mg/L	2 days	0.38% uptake	Dean-Raymond and Alexander, 1976

Table D-2: Effects of NDMA on Birds and Mammals

Species	Route	Dose	Duration	Effects/Endpoint	References
Peking duck	diet	50 mg/kg diet	9 months	anaplastic hemangio-carcinomas	McCracken *et al.*, 1973
foxes	not reported	10 mg/kg body weight		LD_{50}	Koppang *et al.*, 1981
foxes	not reported	0.1, 0.2, 1.0 mg/kg body weight	not reported	hepatic vein lesions, hemangio-sarcomas, haemorrhagic centrilobular necrosis	Koppang *et al.*, 1981
mink	subcutaneous injection	7 mg/kg		LD_{50}	Koppang and Rimeslatten, 1976
mink	diet	0.32, 0.63 mg/kg/d	23-34 days	liver necrosis	Carter *et al.*, 1969
mink	diet	0.18 mg/kg/d		liver necrosis, hemangiomatous liver tumours nephrotoxicity	Martino *et al.*, 1988
mink	diet	0.1 mg/kg/d	321-670 days	hepatic vein lesions, hemangiomatous liver tumours	Koppang and Rimeslatten, 1976

Table D-2 (cont'd): Effects of NDMA on Birds and Mammals

Species	Route	Dose	Duration	Effects/Endpoint	References
mink	diet	0.13-0.15 mg/kg/d	122 days	hepatic vein lesions	Koppang and Rimeslatten, 1976
sheep	diet	0.16-0.7 mg/kg/d	not reported	liver disease	Koppang, 1974
sheep	diet	0.10-0.15 mg/kg/d	> 200 days	no effects	Koppang, 1974
pigs	diet	4.1, 8.2, 20.5 mg/kg/d	64-105 days	hepatic vein lesions, nephrotoxicity	Koppang, 1974
pigs	diet	0.62 mg/kg/d	525 days	no effects	Koppang, 1974
cattle	diet	0.4 mg/kg/d	54 days	hepatic vein lesions	Koppang, 1974
cattle	diet	0.2 mg/kg/d	157 days	hepatic vein lesions	Koppang, 1974
cattle	diet	0.1 mg/kg/d	480 days	hepatic vein lesions	Koppang, 1974

Table D-3: Effects of NDMA on Fish

Species	Route	Dose	Duration	Effects/Endpoint	Reference
fathead minnow	water	940 mg/L	96 hour	LC_{50}	Draper III and Brewer, 1979
zebra fish	water	50 mg/L	22-23 wk	hepatomas	Aydin and Bulay, 1983
guppy	diet	4800 mg/kg	> 13 months	hepatic tumours, leiomyosarcoma of the mesentery	Sato *et al.*, 1973
guppy	water	100 mg/L	56 days	liver tumours	Pliss and Khudoley, 1975
rainbow trout	intraperitoneal injection	1770 mg/kg		LD_{50}	Grieco *et al.*, 1978
rainbow trout	diet	75, 300, 1200, 4800, 19200 mg/kg diet	20 months	adenomas and adenocarcinomas of the liver	Ashley and Halver, 1968
rainbow trout	diet	3, 200, 400, 800 mg/kg	52 weeks	hepatocellular carcinoma	Grieco *et al.*, 1978

Table D-4: Effects of NDMA on Aquatic Invertebrates

Species	Route	Dose	Duration	Effects/Endpoint	Reference
flatworm	water	1365 mg/L	96 hour	LC_{50}	Draper III and Brewer, 1979
scud	water	330 mg/L	96 hour	LC_{50}	Draper III and Brewer, 1979
scud	water	280 mg/l	96 hour	LC_{50}	Draper III and Fisher, 1980
scud	water	310 mg/L	96 hour	LC_{50}	Draper III and Fisher, 1980
scud	water	445 mg/L	96 hour	LC_{50}	Draper III and Fisher, 1980
mussel	water	200 mg/L	51 days	digestive gland and hemopoietic system tumours	Khudoley and Syrenko, 1978
mussel	water	200 mg/L	152 days	digestive gland and hemopoietic system tumours	Khudoley and Syrenko, 1978
mussel	water	200 mg/L	51 days	digestive gland tumour	Khudoley and Syrenko, 1977
crayfish (*Procambarus clarkii*)	water	200 mg/L	6 months	degeneration of antennal gland	Harshbarger *et al.*, 1971

Table D-4 (cont'd): Effects of NDMA on Aquatic Invertebrates

Species	Route	Dose	Duration	Effects/Endpoint	Reference
crayfish (*Procambarus clarkii*)	water	100 mg/L	6 months	hyperplasia of tubular cells in hepatopancreas	Harshbarger *et al.*, 1971
crayfish (*Austropotamobius pallipes*)	injection	2250 mg/kg		LD_{50}	Alibaud *et al.*, 1985
crayfish (*Austropotamobius pallipes*)	intravenous injection ~	5 ug 14C-NDMA	30 minutes	high concentration in abdominal muscle and hepatopancreas	Alibaud *et al.*, 1985

Table D-5: Effects of NDMA on Amphibians

Species	Route	Dose	Duration	Effects/Endpoint	References
frog (*Rana temporaria*)	water	5 mg/L	63 days	hepatocellular carcinomas, hepatoadenomas, hemopoietic system tumours	Khudoley, 1977
frog (*Rana temporaria*)	water	5 mg/L	203 days	hepatocellular carcinomas, hepatoadenomas, hemopoietic system tumours	Khudoley, 1977
frog (*Xenopus borealis*)	water	400 mg/L	52 weeks	tumours of liver (cholangio-carcinomas, hepatocellular cancers) and kidney (adeno-carcinomas, anaplastic cancers)	Khudoley and Picard, 1980
palmate newt	intraperitoneal injection	16 g/kg body weight	6-7 injections over 3-4 weeks	tumours of liver (anaplastic, hepatic cell cancer)	Ingram, 1972

APPENDIX E

ANALYTICAL METHODS FOR MONITORING NDMA

APPENDIX E

ANALYTICAL METHODS FOR MONITORING NDMA

1.0 INTRODUCTION

Nearly all recent low level analytical methods employ a gas chromatograph, coupled with a thermal energy analyzer (TEA), electrolytic conductivity (Hall) detector or mass spectrometer (MS) for detection and quantitative determination of NDMA (Fine, 1982). Historically, the thermal energy analyzer (TEA) has been used most often. However, technical improvements in mass spectrometry and its increased availability over the past ten years have made it the current instrument of choice, especially when both selectivity and sensitivity are required.

2.0 ONTARIO MINISTRY OF ENVIRONMENT SAMPLING PROTOCOL FOR NDMA

2.1 Collection and Storage of Samples

Samples are collected in 1 L amber glass bottles with Teflon-lined caps. If non-amber bottles are used, the samples must be protected from light by wrapping in aluminum foil or placing in a light-tight cardboard box.

The samples should be stored at 4°C. NDMA is stable in an alkaline solution. If the sample is acidic, it is neutralized by the addition of sodium hydroxide.

2.2 Sample Preparation

A wide variety of wet chemical preparation techniques may be used prior to gas chromatographic analysis: standard liquid/liquid extraction (EPA, 1987; Ontario Ministry of the Environment, 1989); distillation (Marinelli *et al.*, 1981); or solid phase extraction (SPE). Solid phase extraction techniques used have included XAD resin (Fan *et al.*, 1978), XE340 resin (Kimoto *et al.*, 1981), carbon (Fine *et al.*, 1975), ion exchange resin (Gough *et al.*, 1977) and celite (Hotchkiss *et al.*, 1981; Scanlan *et al.*, 1990).

At the present time, liquid/liquid extraction is the only technique which provides the required sensitivity. The most common solvent used for this method is methylene chloride. The

extraction efficiency appears to be variable, with the variability increasing with decreasing concentration. At low concentrations (1-100 ng/L) recoveries of only 30% are common.

3.0 INSTRUMENTAL METHODS

3.1 Gas Chromatography/Thermal Energy Analyzer

The thermal energy analyzer (TEA) is a nitrosyl-group-specific detector, utilizing a two-stage catalyzed pyrolysis reaction (Farwell *et al.*, 1981; Fan *et al.*, 1978). Its selectivity for thermally labile nitrosamines is better than 10^7 to 1 versus hydrocarbons, common solvents and other nitrogen-containing compounds such as amines (Thermedics, 1985; Fine *et al.*, 1977). NDMA sensitivity in the order of 10-50 pg can be achieved. Co-eluting nitro-containing compounds and high organic compound backgrounds may cause some detector interference.

3.2 Gas Chromatography/Thermionic Specific Detector

The nitrogen-phosphorus (NPD) and thermionic specific (TSD) detectors are ionization type gas chromatograph detectors, responding specifically to compounds containing nitrogen or phosphorus atoms (Patterson, 1989). Unlike the TEA detector, the NPD and TSD detectors respond to all nitrogen- and phosphorus-containing compounds, resulting in possible interferences from a broader range of organic compounds (Farwell *et al.*, 1981). NPD and TSD detectors have NDMA sensitivities in the range of 50-100 pg.

3.3 Gas Chromatography/Electrolytic Conductivity Detector

Electrolytic conductivity (HECD or Hall) detectors can be used to selectively detect halogen-, sulphur- or nitrogen-containing compounds (Farwell *et al.*, 1981). When operated in the nitrogen mode, the HECD detector shows an NDMA/p-nitrotoluene selectivity ratio of 10^3 to 1, with a sensitivity in the range of 25-50 pg (Penton, 1980; Von Rappard *et al.*, 1976).

3.4 Gas Chromatography/Mass Spectrometry

Gas chromatography/mass spectrometry (GC/MS) is used mainly as a confirmatory technique in the determination of NDMA (EPA, 1989; Hotchkiss *et al.*, 1980). GC/MS is both more selective and sensitive than other types of GC detectors (Sections 3.1 to 3.3). Full scan-GC/MS can give detection limits of 160 ng/L in environmental samples (EPA method 1624, 1989). Using

a single ion monitoring (SIM) technique, detection limits can be reduced further by at least an order of magnitude (Howe *et al.*,1981).

3.5 Gas Chromatography/High Resolution Mass Spectrometry

Gas chromatography/high resolution mass spectrometry (GC/HRMS) is considered the most reliable method for the analysis of NDMA (Crosby, *et al.*, 1976; Kimoto and Fiddler, 1982; Ontario Ministry of the Environment, 1989). It avoids potentially high organic background interference and is the best technique for detection/quantitative determination at the low ng/L level.

GC/HRMS is more sensitive and selective than GC/MS. It is usually used to confirm results obtained by other methods, including GC/MS.

4.0 CURRENT LIMITATIONS IN ANALYTICAL METHODS

Several approaches are being investigated by MOE to develop a rugged analytical method capable of testing for NDMA at ng/L levels. A derivatization method combined with low resolution mass spectrometry holds much promise.

A major difficulty in lowering detection limits is the ubiquitous presence of NDMA in water at the 1 ng/L level. While it is probable that methods can be developed to measure background levels of NDMA, they will rely on the preparation of NDMA-free water.

4.1 Current Limitations in Sample Preparation

The liquid/liquid separatory funnel technique is laborious. A roller method to streamline the extraction of NDMA has been shown to be at least as effective.

Various solid phase extraction techniques have been investigated, but none as yet yield the required sensitivity.

A derivatization/distillation method coupled with low resolution mass spectrometry is under investigation by the Ministry. Future development work may allow detection of concentrations in the 1-10 ng/L range. However, since NDMA appears to be ubiquitous at greater than 1 ng/L, blank reduction may be the limiting factor in lowering detection limits. For instance, if normal background levels are in the range of 1-3 ng/L, blank water with less than 0.1 ng/L NDMA will have to be produced.

4.2 Current Limitations in Instrumentation

High resolution mass spectrometry remains the most selective and sensitive method. Tandem mass spectrometry techniques may be shown to perform as well as the high resolution technique. All GC/MS methods allow isotope dilution techniques to be used, techniques which correct variations in sample recovery.

Although detection limits of 1 pg may be obtained using GC/HRMS, the major limiting factor in determining NDMA at very low levels is blank contamination. Typical NDMA levels found in procedure blanks and field blanks lie between 1 and 3 ng/L. To lower detection limits to these levels, more rigid QA/QC would have to be implemented, including glassware and blank water checks and travelling blanks.

5.0 CONCLUSIONS

The current method of choice is the liquid/liquid extraction of samples using methylene chloride, followed by gas chromatography and high resolution mass spectrometry. This allows for a reporting limit of 5 ng/L or 5 parts per trillion (ppt).

APPENDIX F

MATHEMATICAL MODELS USED TO ESTIMATE NDMA CANCER POTENCY

APPENDIX F

MATHEMATICAL MODELS USED TO ESTIMATE NDMA CANCER POTENCY

1.0 U.S. EPA RISK ASSESSMENT

The U.S. Environmental Protection Agency did a risk assessment on NDMA using the data from the Peto study (EPA, 1988; Peto *et al.*, 1984). Data from the study on incidence of liver tumours of all types in female rats were shown to follow the relationship:

$$CI = 51.45\ (d + 0.1)^{6} \cdot t^{7}$$

where,

CI = cumulative incidence
d = dose (mg/kg/day)
t = time in years

After correcting for background response (EPA, 1980), the increased risk of 1 ug/kg/day for 3 years was found to be 7.8×10^{-3} or a slope factor for rats of 7.8/mg/kg/day. The slope factor for humans was calculated by using a body weight correction factor: the cube root ratio of the assumed human body weight of 70 kg and the reported rat weight of 250 g. The human slope factor was found to be 51/mg/kg/day (EPA, 1988).

The risk estimates are summarized in Table F-1.

The EPA points out that unit risk should not be used if the water concentration exceeds 7 ug/L or if the air concentration exceeds 0.7 ug/m^3. Above this concentration the slope factor may differ from that stated.

Table F-1. Summary of Risk Estimates

Drinking Water Concentrations at Specified Risk Levels

Risk level	Concentration
10^{-4} (1 in 10,000)	7×10^{-2} ug/L
10^{-5} (1 in 100,000)	7×10^{-3} ug/L
10^{-6} (1 in 1,000,000)	7×10^{-4} ug/L

Drinking Water Risk -- 1.4×10^{-3}/ug/L

Air Concentrations at Specified Risk Levels

Risk level	Concentration
10^{-4} (1 in 10,000)	7×10^{-3} ug/m^3
10^{-5} (1 in 100,000)	7×10^{-4} ug/m^3
10^{-6} (1 in 1,000,000)	7×10^{-5} ug/m^3

Inhalation Unit Risk -- 1.4×10^{-2}/ug/m^3

2.0 CDHS RISK ASSESSMENT

The California Department of Health Services estimated the cancer potency of NDMA using the same data (CDHS, 1988; Peto *et al.*, 1984).

Following an earlier work, Peto chose to describe cumulative tumour incidence (CI) after t years of treatment by the expression: (Peto and Lee, 1973; Peto *et al.*, 1984)

$$CI(t) = a\,(d - x)^m\,t^7 \qquad (1)$$

where,

CI = cumulative incidence
d = dose (mg/kg/day)
t = time in years

Thus, the cumulative risk of cancer varies directly with the seventh power of the duration of exposure.

At low doses, the most sensitive site was the liver. For the liver, the dose response relationship was highly non-linear when high to low dose groups were taken together. However, at low doses it was found to be consistent with a linear increase of incidence with dose (estimates of x = 0.1, m = 6 for both males and females). Peto noted that the existence of a moderately high background made low-dose linearity more probable, at least in the range of doses that produced extra effects that did not greatly exceed this background.

Estimates of a in Equation (1) were given as 51.45 for females and 37.43 for males. Using these values of a, x and m, Peto found that at doses of 1.0 ug/kg/day the risk of NDMA-induced tumours would be about 0.03 - 0.04% at two years. In the presence of other causes of death, lifelong exposure from week 6 onwards would probably have a risk seven times greater.

To estimate cancer potency or the slope of the dose response curve at low doses, the derivative of CI(t) with respect to the dose d was taken:

$$d[CI(t)]/dd = a\ t^7\ [6x^5 + 30x^4d + 120x^3d^3 + 30x^22d^4 + 6xd^2 + d^5]^1$$

For small doses, this simplifies to:

$$Q = 6\ a\ t^7\ x^5 \qquad (2)$$

Peto found Q to be 0.4/mg/kg/day for female rats and 0.29/mg/kg/day for male rats. This potency value is consistent with the statement that risks from doses of 0.001 mg/kg/day over two years of treatment would range from 0.03% (0.39 x 0.001) to 0.06% (0.4 x 0.001). However, Peto indicated that lifelong exposure (beyond 2 years) would result in risks about seven times as large. The median natural lifespan for low dose and control animals in the study was considerably larger than two years. Thus, the potency estimates of 2.8 (0.4 x 7) and 2.0/mg/kg/day for female and male rats, respectively, are more appropriate estimates of potency (Peto *et al.*, 1984).

Human potency estimates were obtained from the equation:

$$q_{human} = q_{animal} \cdot (bw_h/bw_a)^{1/3} \qquad (3)$$

Assuming female and male rats weighed about 250 and 450 grams, respectively, these correspond to human potency estimates (q_{human}) of 16 and 12/mg/kg/day.

If Peto's values from Equation (1) are used in Equation (2), one obtains a Q value of 6.8/mg/kg/day ($6(51.45)(3^7)(0.1^5)$). Applying the 6.5 scaling factor, the resulting human potency estimate of 44.2/mg/kg/day was obtained. This suggests the EPA and California values are comparable.

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[1] this should read:

$$t^7\ [6x^5 + 30x^4d + 120x^3d^2 + 30x^22d^3 + 30xd^4 + 6d^5]$$

•

3.0 ESTIMATES USING THE LINEARIZED MULTISTAGE MODEL

The "multistage" polynomial model, originally described in 1961, adequately fits a large body of human and animal bioassay data (Armitage and Doll, 1961.) Subsequent modification has made it the low dose extrapolation method of choice for the U.S. EPA (Crump and Watson, 1979; Anderson *et al.*, 1983).

If *P(d)* represents the lifetime risk or probability of cancer at dose, d, then:

$$P(d) = 1 - \exp[- (q_0^* + q_1 d + q_2 d^2 + \ldots + q_k d^k)]$$

where,

q_i is greater than 0, and i = 0,1,2,....,k

The model employs enough arbitrary constants (q_i) so as to fit almost any set of monotonically increasing dose response data. The parameter q_0 represents the background cancer incidence of the tumour. The point estimate of coefficient q_i at any dose d is calculated by estimating the upper 95% confidence limit of the data.

The cancer potency or slope factor is defined as q_1^*, the upper 95% confidence limit on q_1. q_1^* is expressed in units/mg/kg/day. These parameters can be estimated using the GLOBAL series of computer programs, now called ToxRisk, developed by Crump (Crump *et al.*, 1989).

ToxRisk was employed to estimate q_1^* using the BIBRA data for total liver tumour incidence in female rats, excluding hyperplastic nodules. ToxRisk was run for either all doses or with the upper doses dropped so as to use the lower linear part of the dose response curve. The program was run with the surface area to body weight conversion factor incorporated. Thus, the q_1^* are human slope factors.

The q_1^* values obtained were comparable to the U.S. EPA Weibull method. The q_1^* for total liver tumours, based on female rat data, ranged up to 37.16/mg/kg/day depending on how the dose response curve was fitted.

LIST OF ABBREVIATIONS

LIST OF ABBREVIATIONS

asn - asparagine

AMMN - acetoxymethylmethylnitrosamine

ATSDR - Agency for Toxic Substances and Disease Registry

AFID - Alkaline flame ionization detector

BIBRA - British Industrial Biological Research Association

BCF - bioconcentration factor

b.w. - body weight

CHO - Chinese hamster ovary

C.I. - cumulative incidence

CDHS - California Department of Health Services

DNA - deoxyribonucleic acid

DMNA - dimethylnitrosamine

DMN - dimethylnitrosamine

DMA - dimethylamine

EC_{50} - median effective concentration

EPA - Environmental Protection Agency (U.S.)

FDA - Food and Drug Administration (U.S.)

g - gram

GC/MS - gas chromatography/mass spectrophotometry

HGPRT - hypoxanthine guanine phosphoribosyltransferase locus

HSDB - Hazardous Substances Databank

HPLC - High Pressure Liquid Chromatography

HRMS - high resolution mass spectrophotometry

i.v. - intravenous administration

i.p. - intraperitoneal administration

IARC - International Agency for Research on Cancer

Kow - octanol/water partition coefficient

L - litre

LD_{50} - dose lethal to 50% of recipients

LC_{50} - concentration lethal to 50% of recipients

LMS - Linear Multistage

MOE - Ontario Ministry of Environment

MCPA - 2-Methyl-4-chloro-phenoxyacetic acid

mL - millilitre

mM - millimolar

mg - milligram (10^{-3} g)

NTP - National Toxicology Program

ng - nanogram (10^{-9} g)

nm - nanometer (10^{-9} metre)

NDMA - N-nitrosodimethylamine

NPYR - N-nitrosopyrrolidine

NPIP - N-nitrosopiperidine

NIEHS - National Institute of Environmental Health Sciences (U.S.)

NIOSH - National Institute for Occupational Safety and Health

NPRO - N-nitrosoproline

na - not available

nd - not detected

ppm - parts per million

ppb - parts per billion

ppt - parts per trillion

Q-FLOC - quaternary ammonium polyelectrolyte

QRA - quantitative risk analysis

RTECS - Registry of Toxic Effects of Chemicals Substances

2,4-D - 2,4-dichlorophenoxyacetic acid

TEA - Thermal Energy Analyzer

TMA - trimethylamine

UDS - unscheduled DNA synthesis

ug - microgram (10^{-6} g)